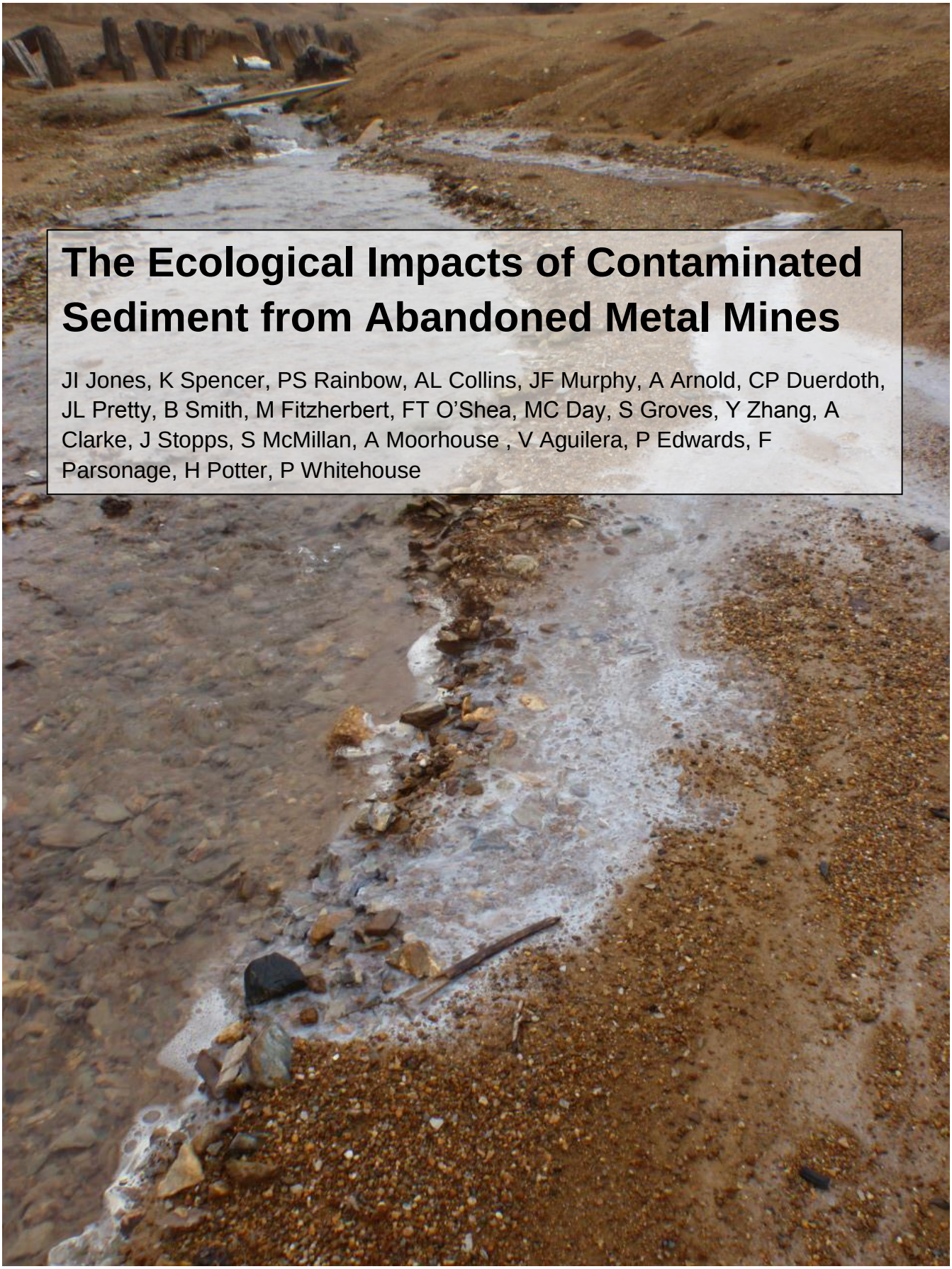

The Ecological Impacts of Contaminated Sediment from Abandoned Metal Mines

JI Jones, K Spencer, PS Rainbow, AL Collins, JF Murphy, A Arnold, CP Duerdoth, JL Pretty, B Smith, M Fitzherbert, FT O'Shea, MC Day, S Groves, Y Zhang, A Clarke, J Stopps, S McMillan, A Moorhouse , V Aguilera, P Edwards, F Parsonage, H Potter, P Whitehouse

Final report

of

WT0970 Characterisation and targeting of measures for (non-coal) polluted mine waters – Impacts of contaminated sediment on ecological recovery

September 2016

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Executive Summary

Pollution from abandoned non-coal (i.e. metal) mines is a serious impediment to rivers meeting the water quality targets set out in River Basin Management Plans. Recent work has identified the mines most likely to be causing a significant environmental impact and hence where efforts to prevent pollution need to be focussed. Yet, it is not clear to what extent rivers, and the animal and plant life they support, are impacted by the legacy of past pollution still bound up in river sediments. Work will be undertaken to reduce toxic metals in mine waters before they enter the river. However, if riverbed sediments are already contaminated and affecting life in rivers, the planned clean-up of mine water sources may not result in recovery of ecological condition.

A controlled laboratory experiment was undertaken where river invertebrates (mayfly larvae) from an uncontaminated site were incubated with contaminated riverbed sediment collected downstream of an abandoned metal mine. Concentrations of metals in the tissues of the mayflies increased over the duration of the incubation, particularly those metals that were in high concentrations in the sediment, i.e. cadmium, copper and zinc. As the sediment was the only substantial source of metals in the experiment, it is apparent that the contaminated riverbed sediment was acting as a source of bioavailable metals. It is likely that contaminated sediments, including riverbed sediment, will act as a source of bioavailable metals, at least to benthic organisms, even where mine drainage water is treated to reduce metal concentrations.

Metal toxicity occurs when the rate of metal uptake into an organism exceeds the combined rates of excretion and physiological detoxification. Current tests of metal toxicity on biota typically do not match in scale (temporal, spatial and taxonomic range) with assessments of ecological quality undertaken for management, which raises questions regarding the adequacy of environmental limits based on laboratory testing.

Existing data were compiled describing geochemistry of riverbed sediment and the Biological Quality Elements invertebrates, diatoms, macrophytes and fish, collected by the regulatory authorities to assess the condition of rivers. As toxic effects of trace metals were not expected at low concentrations, the biological response to sediment metal concentration was determined using a threshold model. Thresholds were found for biotic metrics based on species richness, but other metrics (diatom EQR, macrophyte EQR and invertebrate ASPT) displayed implausible positive relationships with sediment metal concentrations and should not be relied upon for classification of ecological status in waterbodies affected by mining.

New data were collected from 20 spatially-independent river catchments in areas affected by metal mine facilities, including samples of the macroinvertebrate community, bioavailability of metals (assessed as metal concentrations in the body tissue of tolerant taxa), and sediment metal concentrations. There were strong correlations between sediment metal concentrations and measured bioavailability, particularly for copper and lead. Measurements of bioavailable metals were related to changes in taxon richness in the invertebrate samples. The data were used to develop a new biotic index (MetTol), which can be used to assess the extent of ecological damage from metal contamination using standard invertebrate monitoring data, and to construct dose response curves based on species sensitivities.

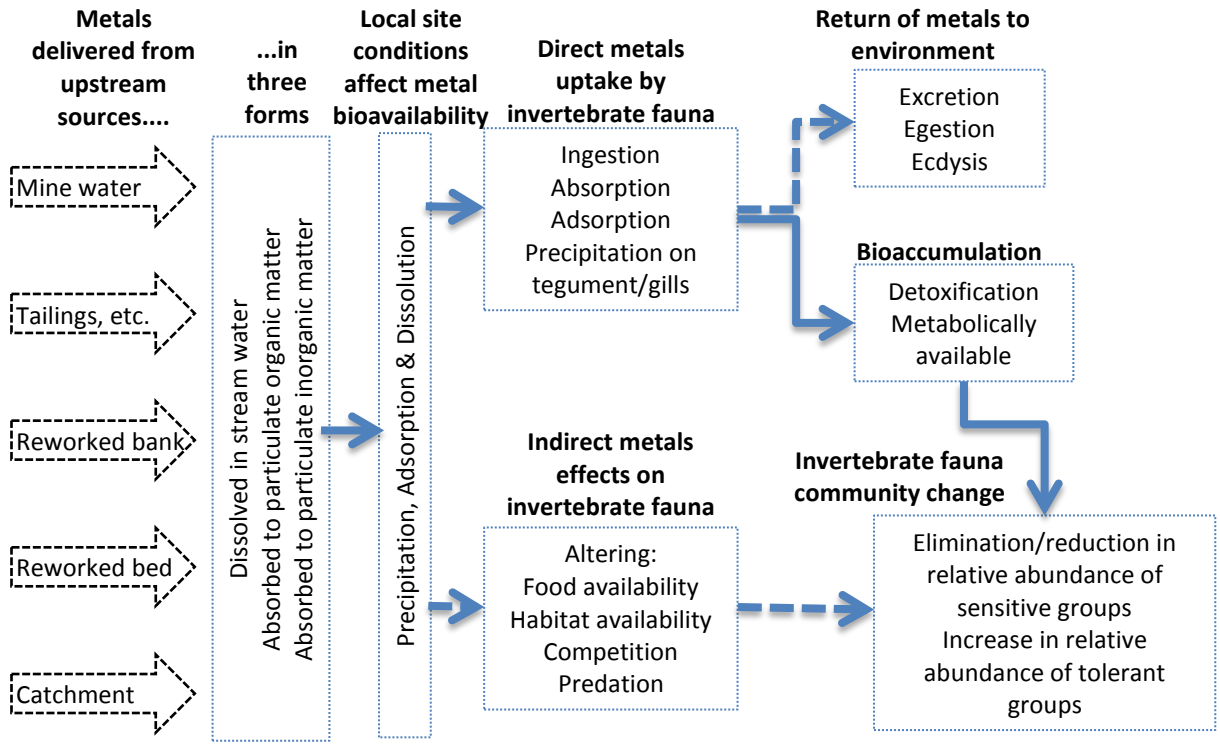
A number of approaches were used to establish tolerable limits for sediment metal concentrations based on ecological data, and the results compared with existing Canadian sediment quality guidelines. The limits for copper derived from ecological data were most consistent with existing sediment guidelines. The limits for other metals (silver, arsenic, cadmium, nickel, lead and zinc) derived from ecological data were up to an order of magnitude above the Canadian interim sediment quality guidelines. These existing guidelines, based on toxicological data, may be too precautionary, and we suggest that guideline sediment concentrations based on ecological data may provide a more appropriate level of protection for the environment.

Non-technical Summary of Key Findings

Pollution from abandoned non-coal (i.e. metal) mines is a serious impediment to rivers in England and Wales meeting the water quality targets set out in River Basin Management Plans. Recent work has identified the mines that are most likely to be causing a significant environmental impact and hence where efforts to prevent pollution needs to be focussed. However, it is not clear to what extent rivers, and the animal and plant life they support are impacted by the legacy of past pollution still bound up in river sediments and to what extent the problem is linked to metal-contaminated material currently entering rivers from mines and mine waste heaps. River basin managers will focus on the most damaging sites to clean up mine water (removing or reducing toxic metals) before it enters the river but, if the sediment in the river bed is already contaminated and affecting life in rivers, the planned clean-up of mine water sources may not result in the expected recovery of ecological condition. The aim of this project is to improve our knowledge of the impact of metal-rich sediments upon river life and to investigate whether these sediments will inhibit ecological recovery following remediation of mine water discharges.

Metals from mining sources are delivered to rivers in various forms (dissolved and particulate) via a variety of routes. Not all the metal present will be in a form that is biologically available (bioavailable) to cause toxicity, and the conditions at the river site strongly influence the proportion that is. A review of existing evidence regarding the bioavailability of metals indicated that current understanding and models are based solely on uptake of dissolved metals from the water. Evidence exists for alternative routes of uptake from the sediment, via particulate material taken up in the diet of benthic organisms. Assumptions of uptake via direct contact between benthic organisms and sediment pore water do not reflect biological processes.

Schematic diagram of how metals derived from mine workings and the catchment impact the invertebrate community



Given the lack of appropriate models of bioavailability of metals from sediment, a simpler approach may be to use body concentrations of metals in tolerant taxa that accumulate trace metals in their tissues as a direct measure of the bioavailable fraction.

Trace metals are toxic as a result of their chemical properties, especially their affinity for the elements sulphur and nitrogen, which are present in the make-up of many biological macromolecules vital to the metabolism of plants and animals, especially in proteins, e.g. enzymes. Organisms have evolved physiological mechanisms to prevent a build-up of all potentially toxic metals in a form that could cause toxcity. Toxicity occurs when the total rate of metal uptake into an organism exceeds the rate at which the metal is excreted or physiological detoxified.

Establishing safe environmental limits that are relevant to field conditions presents a challenge. This is because the data used to set the limits relies on single species toxicity testing in the laboratory, which can be very different to the spatial scale encountered in the field, the range of species exposed and the length of time that animals are exposed. This matters because current water management legislation focuses on community-level biological impacts at the waterbody scale. Therefore, there is a discrepancy between the way we derive acceptable limits for metals and the way we assess ecological quality. Tools are needed that allow us to assess community level damage from metals in order to predict the consequences and cost-effectiveness of water body management measures. A weight of evidence approach to the ecotoxicological assessment of mining-affected stream sediments is recommended.

Existing data describing geochemistry of riverbed sediment were compiled from British Geological Survey's Geochemical Baselines Survey of the Environment (G-BASE) project, and the Biological Quality Elements (BQE) invertebrates, diatoms, macrophytes and fish, collected using the field sampling methods used by the regulatory authorities to assess the biological condition of rivers. Biological data were matched spatially to data describing sediment chemistry in ArcMap 10.2 using the river network. Biology and chemistry sites were matched if they were both on the same section of river channel, without any inflows between them. Once sites had been matched spatially, the BQE sampling year that represented the best temporal match with the chemical sampling occasion was chosen. A total of 2,833 sites were identified with matching sediment chemistry and biology.

It is likely that metals occur together, making it difficult to ascribe impacts to a particular metal. To overcome this, initial analysis of the G-BASE data involved a pairwise comparison of the metals silver, cadmium, chromium, copper, nickel, mercury, lead, tin and zinc, and the metalloids arsenic and antimony, to establish when these occur together and, hence, identify any potential confounding effects that should be considered when interpreting relationships between chemistry and biology. Arsenic, cadmium, copper, nickel, lead and zinc tended to co-occur, but there was sufficient variation to enable the influence of each element to be assessed independently.

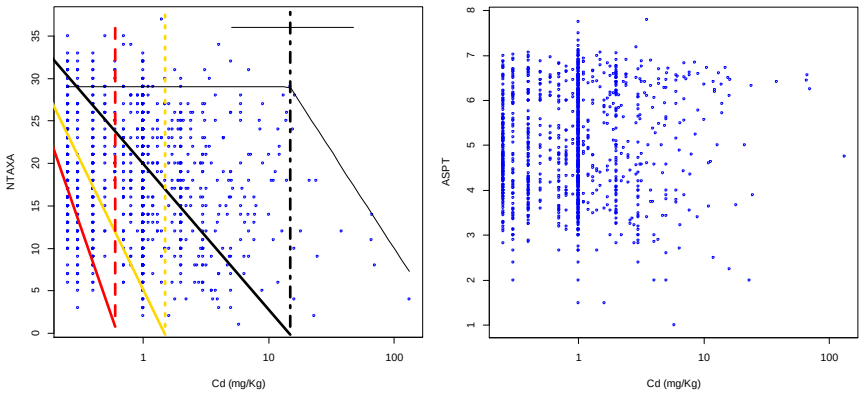
To understand better the range and distribution of concentrations of metals and metalloids in stream sediments, a frequency distribution of measured metal concentrations was constructed from the G-BASE data for each element. The frequency distribution of sites were compared with the Canadian interim sediment quality guidelines (Canadian Council of Ministers of the Environment, 1999) and the Australian and New Zealand Low Trigger Value

(ANZECC and ARMCANZ, 2000) where available. In this way, the proportion of UK sites exceeding these sediment quality guidelines could be estimated.

The toxic effects of trace metals are not expected to occur until the uptake rate has exceeded the combined rates of efflux and detoxification. Hence, we would expect a threshold biological response to sediment metal concentration, below which there was no influence of sediment metal content. We used a technique called quantile regression to fit a threshold response to plots of measured biology and chemistry. Matched datasets describing the response of invertebrates, macrophytes, diatoms and fish to sediment chemistry were analysed for the elements antimony, arsenic, cadmium, chromium, copper, iron, lead, nickel, silver, tin and zinc. For both invertebrates and diatoms the number of taxa provided a better response to sediment metal concentrations than did the other classification metrics tested, such as diatom EQR, macrophyte EQR and invertebrate ASPT.

The modelled thresholds estimated in this study were compared with the Canadian interim sediment quality guidelines (Canadian Council of Ministers of the Environment, 1999) and the Australian and New Zealand Low Trigger Value (ANZECC and ARMCANZ, 2000) which are both largely based on data from laboratory trials. Threshold concentrations varied over the different BQEs. For copper the existing environmental standards were comparable to the findings here, with threshold responses at relatively consistent concentrations across invertebrates (NTAXA, EQR NTAXA), diatoms (No. Taxa, TDI) and macrophytes (No. Aquatic Taxa). For invertebrates (NTAXA) the threshold concentrations for lead, arsenic and zinc were close to the Australian and New Zealand Low Trigger Value. For other elements, the modelled thresholds were typically an order of magnitude greater than existing sediment quality guidelines.

Despite the uncertainty involved in the data matching exercise used to produce the datasets, these findings based on existing field data suggest that several of the existing sediment quality guidelines may be too precautionary, at least for fish and invertebrates.



***Response of the invertebrate indices NTAXA and ASPT to sediment Cd concentration. Black dashed line = modelled threshold, red and yellow lines =* existing Canadian and Australia/NZ guidelines.**

The next step was to relate the response of the benthic macroinvertebrate community to the bioavailability of metals at sites where the sediment has been contaminated by mining. This was done by collecting new chemical and biological data from 20 separate river catchments in areas affected by non-coal mine facilities. Within each catchment, five sites were visited comprising an upstream control site, a site immediately downstream of the metal mine

facility, a site on the impacted stream further downstream in an erosional reach, and a site further downstream on the impacted stream in a reach where sediment tends to be deposited (but staying upstream of major urban areas), and an additional control site on a unimpacted watercourse nearby.

At each site, four different types of data were collected:

i) the macroinvertebrate community,
ii) biomonitor taxa: body concentrations of metals in these tolerant taxa were used as a direct measure of bioavailability,
iii) the metal content of the fine sediment in the stream bed, and
iv) the amount of fine sediment in the stream bed.

At the most downstream site a larger sample of the well-mixed fraction (< 63 µm) of fine-grained sediment was also collected to determine the relative contributions of catchment sources, including mine waste, to the riverbed sediment.

There were clear regional differences in the metal content of river sediments, with higher copper and tin concentrations in the southwest, and higher cadmium, lead and zinc concentrations in Wales and the north of England. These differences reflect regional differences in geology. Sites were sampled where sediment concentrations of copper, chromium, lead, nickel and zinc ranged either side of existing sediment quality guidelines, indicating that, as expected, the sediment at the targeted mine-impacted sites was likely to be causing ecological effects. However, the sediment concentrations of cadmium were in excess of existing sediment quality guidelines at the majority of sites sampled. This suggests either that cadmium is a widespread issue within these mine-impacted sites or that the sediment quality guidelines for cadmium may be overly precautionary.

Within the organisms used to determine bioavailable concentrations ('biomonitor' species: metal-tolerant species such as *Baetis*, *Gammarus, Leuctra and Rhyacophila)* there were strong correlations between the body concentrations of various metals, reflecting

a) the co-occurrence of certain metals due to geology,
b) variation in bioavailability due to local conditions, affecting all metals present at a site,
c) behaviour of metals, particularly with regards physiology (e.g. cadmium can act as a surrogate for zinc).

Strong correlations were generally apparent between the body concentrations of metals in *Baetis* (the most frequently encountered species) and the other species collected as biomonitors (with the exception of *Gammarus*).

Whilst local conditions may influence the bioavailability of metals, the body concentrations of metals in the biomonitor species were expected to be correlated with the metal concentrations in the sediment. The extent to which the body and sediment concentrations are correlated will depend on the bioavailability of the metal, and the physiology of the organism. Copper and lead showed the strongest relationships between body and sediment concentrations, significant for all four species tested *Baetis, Hydropsyche, Leuctra* and *Rhyacophila*. Significant relationships were also found for nickel and zinc, although not for all biomonitor taxa. The residuals of the relationships between sediment and body concentrations of copper and lead were significantly negatively correlated with the pH measured at the time of sampling, indicating an influence of pH (or a correlated variable) on bioavailability. Higher pH was associated with reduced bioavailability.

Further significant ($p \leq 0.05$) or close to significant ($p \leq 0.1$) relationships were found between the concentrations of lead, copper and arsenic in the fine fraction of sediment (< 63 µm) and corresponding tissue concentrations in *Baetis*, *Hydropsyche* and *Rhyacophila* (Figure 4.6). There was also a significant relationship between cadmium concentration in the fine fraction of sediment and in the tissues of *Hydropsyche*, and a close to significant relationship for zinc concentrations in *Hydropsyche.*

The relationships between sediment metal concentrations and corresponding tissue concentrations support the use of biomonitor taxa to assess bioavailability and indicate that riverbed sediments may be a substantial source of bioavailable metals.

Mean dissolved concentrations of copper, lead, zinc and cadmium in river water were correlated with corresponding concentrations in the fine sediment. These results suggest that:

a) the abandoned metal mines targeted in this project were releasing both particulate and dissolved metals into the environment, with the extent of release of both forms dependent on the extent of contamination at each site, and/or
b) metals in river water and sediment are not independent. Rather, metals can move between these two compartments.

We investigated the relationship between invertebrate diversity and bioavailability of metals, as measured using the body concentrations in biomonitor taxa. *Baetis* provided significant relationships between body concentrations and invertebrate diversity for arsenic, cadmium, copper and zinc, and a close to significant relationship for lead. However, the threshold for the model with zinc was near to the upper limit of the range of body burden measured and may have been trivial (i.e. highly influenced by the few points above the threshold). *Hydropsyche* provided significant relationships for copper and nickel, and a close to significant relationship for lead. *Leuctra* provided significant relationships for arsenic, cadmium, copper, nickel and zinc. *Rhyacophila* only provided a close to significant relationship for lead. These results confirm that the biomonitor approach can be used to assess the bioavailability of metals from sediment at ecologically significant concentrations. Threshold concentrations in the bodies of the biomonitor species were converted into sediment metal concentrations using the relationships between metals in sediments and biomonitor tissues. This leads to sediment thresholds that could provide safe limits, although the two-step process increases uncertainty in the derived values.

Ordination techniques were used to determine the influence of the metal stress gradient on macroinvertebrate community composition, whilst accounting for natural background variation among stream types. Using this approach, the relative sensitivity to metals stress of a range of macroinvertebrates was quantified and used as the empirical basis for a new species-level Metal Tolerance (MetTol) biotic index.

The MetTol index can be applied to standard invertebrate monitoring data to assess the extent of ecological damage due to metal contamination at test sites. In this way the importance of metal contamination as a cause of ecological degradation at a site can be assessed. The average index value across all scoring taxa provides an assessment of damage at a site.

The MetTol biotic index was tested both using the calibration dataset and with independent data (using the data of Bass et al. 2008). MetTol was significantly correlated with metal stress, and particularly maximum standardised metal concentration. We also related other routinely used biotic indices (WHPT-ASPT, WHPT-NTAXA and AWICsp) to the two measures of metal stress and to MetTol to assess whether it offered additional explanatory power over the *status quo*, and found that MetTol was consistently better related to metal stress than the other indices.

To give some insight into the relative importance of water and sediment as sources of bioavailable metals, we compared the relationship between concentrations of metals in the tissues of the biomonitor taxa used here (our data) and *Leuctra* (existing data), and the corresponding metal concentrations in the sediment and stream water.

Pronounced relationships between body metal concentrations and sediment metal concentrations were found, suggesting that the sediment is an important source of bioavailable metals. However, metals in river water and sediment are not independent, and both compartments may act as a source of bioavailable metals.

Although sediments appear to be an important source of bioavailable metals, we were unable to fully partition the relative importance of water and sediment using these field data: further experimental work will be required to determine the relative importance of water and sediment.

Overall, the independent testing confirmed that the new MetTol index is sensitive to bioavailable metal contamination, whether it is determined from analysis of tissue concentrations in selected biomonitor taxa, predicted from stream water chemistry or inferred from metal concentrations in the bed sediment.

Based on the field data describing the response of species to bed sediment metal concentrations, wwe sought to identify threshold concentrations beyond which river macroinvertebrate communities are likely to be harmed by a given metal. Twenty-four taxa were selected to represent the full range of responses to each of cadmium, copper, nickel, lead and zinc, from the most sensitive taxon to the most tolerant taxon. Dose-response curves were derived based on these data. Thresholds were determined in two ways. First, we used the approach to deriving ecological effect concentrations described by de Deckere et al. (2011) to calculate the Lowest Effect Level (LEL) and the Severe Effect Level (SEL). Second, we used species sensitivity distributions (SSD) to identify the Hazardous Concentration that would affect 5%, 10% and 20% of taxa (HC_5, HC_{10}, HC_{20}).

A number of approaches have been used in this report to establish safe limits for sediment metal concentrations based on ecological data. Broadly, the approaches fall into three classes, a) approaches based on species sensitivities established through ordination of newly collected field data, b) a threshold derived from species richness and measured bioavailability (as body burden), in turn translated into a sediment concentration, and c) thresholds based on relationships between G-BASE sediment metal concentrations and EA/NRW biological monitoring data. The values derived using these various approaches, and existing sediment quality guidelines (SQG), were compared. The safe limits derived from ecological data most consistent with existing sediment guidelines were for copper. The safe limits for other metals (cadmium, nickel, lead, and zinc) derived from ecological data

were approximately two to four times the ANZECC and ARMCANZ low trigger value, and up to an order of magnitude above the Canadian interim sediment quality guidelines.

The existing guidelines, based on toxicological data, may be too precautionary, and we suggest that guideline sediment concentrations based on the species sensitivities derived from ecological data may provide a more appropriate level of protection for the environment.

Summary of thresholds for sediment metal concentrations (mg kg^{-1}) based on ecological data.

	Existing Safe Limits		Based on Ecological Data					**Suggested Threshold**
	Canada°	Australia and NZ*	SEC†	SSD	Biomonitor body burden	Quantile regression G-BASE data		
	Interim SQG	Low Trigger Value	LEL	HC_{10}	Mean threshold	Geometric mean of Nº Taxa	Lowest of Nº taxa	
Ag		1				7.9	7.9	**7.9**
As	5.9	20			384	59.6	27	**27**
Cd	0.6	1.5	4.7	4.9		6.8	4	**4.7**
Cu	35.7	65	37.9	34.3	75	86.2	32.6	**34.3**
Ni		21	39.8	39.8		109	41.4	**39.8**
Pb	35	50	133	220	54	295	49.6	**133**
Sn						17.4	9	**9**
Sb		2				5	1.8	**1.8**
Zn	123	200	447	498	1,561	849	286	**447**

° Canadian Council of Ministers of the Environment (1999)
* Australian and New Zealand Environment and Conservation Council, Agriculture and Resource Management Council of Australia and New Zealand (2000)
† Sediment Effect Concentration

In order to gain an insight into the sources of metals arriving at the point of impact, a process called sediment source apportionment was undertaken. For each of the 20 catchments, potential fine-grained sediment source types were identified and sampled, which included agricultural (grass and arable) topsoils, damaged road verges, channel banks/subsurface sources, urban street dust and the abandoned metal mines. Mine waste samples were primarily collected from slag heaps, spoils heaps and tailings as appropriate, depending on the structure of the mine site and ease of accessibility. A combination of statistical discrimination techniques and mass balance numerical modelling was used to derive estimates of relative contributions to the fine-grained riverbed sediment. Robust estimates were derived for all catchments except one, Rea Brook, Shropshire.

Mine waste contributed between 3% (River Greta) and 26% (Red Tarn Beck) of the fine-grained riverbed sediment. Within this overall range, high relative contributions were also predicted for the River East Allen (19%), Hayle (18%), Egglestone Beck (16%) and Bolingey Stream (16%). Fine-grained sediment inputs from the abandoned mine workings at the remaining sites were lower, reflecting a combination of the coarse grain-size of the mine workings/waste, the development of protective vegetation cover and the introduction of

pollution mitigation measures including settling ponds. Despite mine waste comprising a relatively small proportion of riverbed sediment, ecological impacts were observed at many sites.

Large quantities of contaminated mine sediment do not have to enter the river system to cause adverse effect on the local ecology.

A laboratory experiment was undertaken in replicated aquaria (mesocosms) where a chosen biomonitor species (*Baetis* spp) and overlying water were collected from an unimpacted source (Dean Burn) and incubated with contaminated riverbed sediment (River Mardle) under controlled conditions. The aim was to establish the influence of the gradients of environmental conditions on the bioavailability of metals. The only source of any metals accumulated by the *Baetis* was the sediment, either directly or indirectly.

The body burden of all metals in *Baetis* increased over the duration of the incubation, compared with the starting condition, particularly those metals that were in notably high concentrations in the sediment and *Baetis* collected from the River Mardle during the field survey (i.e. arsenic, cadmium, copper and zinc). At the end of the experiment the body burdens of cadmium, copper and zinc were in excess of, or close to concentrations considered high for *Baetis*. The body burdens of cadmium, copper and zinc were significantly (MANOVA $p = 0.0018$) higher in *Baetis* from those treatments that had larger amounts of organic matter added to the sediment.

It was clear that the experimental treatments influenced the partitioning of metals in the sediment, particularly resuspension. However, in contrast with the effects on bioavailability, organic matter had little influence.

This disparity between the influence of the experimental treatments on bioavailability and on the behaviour of metals in the sediment suggests that the uptake of metals by biota is influenced by some factor other than the chemical behaviour of the metals. We suggest that this is most likely to be biological, i.e. the consumption of metals through the diet.

As both the water and *Baetis* used in the experiment were from an uncontaminated site, the Dean Burn, the sediment was the only substantial source of metals. It is apparent that the contaminated sediment from the River Mardle was acting as a source of bioavailable metals in this experiment.

From this we can conclude that it is likely, even where mine drainage water is treated to reduce metal concentrations, contaminated sediments, including river bed sediment, will act as a source of bioavailable metals.

Simple rules to identify where contaminated sediments pose the greatest risk are outlined based on,

a) the concentration of contaminants in the source material,
b) the delivery of contaminated sediment to the site,
c) the retention of contaminated sediment at the site,
d) the influence of environmental conditions at the site on bioavailability of metals from contaminated sediment.

These rules were summarised in a checklist of yes/no criteria to identify the risk that sediment from abandoned metal mines presents to the ecology at a river site.

The project produced the following tools:

- Sediment quality threshold concentrations based on ecological data.
- An assessment of the utility of current biological indices for detecting metal stress.
- A biotic index, MetTol, to be used with routine invertebrate monitoring data to assess the extent of ecological damage due to metal contamination.
- A checklist for identifying river sites where the risk of sediment from abandoned metal mines to the ecology is enhanced.
- Guidance on future research needs.

1 How do Metal-rich Sediments Derived from Mining Impact Freshwater Ecology?

Objective 1a

Review the existing published and grey literature to provide a fully informed platform from which the project can proceed. The review would have a particular focus on collating evidence of biological impacts linked to trace metal contamination of sediment and will update previous reviews, capturing recent developments in the field.

1.1 Introduction

Metal-rich sediments have the potential to impact upon life in freshwater streams and rivers, and, thereby, to inhibit recovery of ecological condition after any remediation of mine water discharges. The extent to which such metal-rich sediments are causing impacts is as yet unknown. Metal-rich sediments by definition consist of sediments with high loadings of trace metals, all of which are toxic to biota above a threshold level. In mining impacted rivers, metals tend to be very strongly associated with sediment and these high affinities mean that these metal-rich sediments do not give up their associated metals very easily, even when relatively metal-free water is flowing over them, for example after remediation. Thus, the sediments remain metal-rich over long time periods and have long-term potential ecotoxicological interactions with local biota, unless the sediments themselves are physically removed downstream by strong water flow to be replaced by new less metal-rich sediment.

The composition of river bed sediments will influence biota even in the absence of metal contamination (Kemp et al. 2011; Jones et al. 2012a, b, 2014), and it is necessary in any investigation of the effect of metal-rich sediments on biota to tease out the separate effects of any modification of bed sediments *per se* from the effects of any sediment-associated metals. Furthermore, it necessary to separate the effects of any sediment-associated metals from any other effects associated with mining activities (low pH water, channel modification, industrial pollutants) and the development of mining communities (organic pollution).

This review aims to provide an informed platform on the assessment of how metal-rich sediments impact on freshwater stream life, incorporating recent developments in the field, focusing on biological impacts linked to trace metal contamination of sediment. Laboratory-derived environmental quality standards are difficult to apply to the field situation as many complicating factors exist in the real world, such as physiological acclimation, water chemistry, metal interaction effects, and particularly the ingestion of sediment by deposit-feeding biota which are at particular ecotoxicological risk in the presence of metal-rich sediments (Environment Agency, 2009). Thus, as alternatives to laboratory-derived standards, there is a strong case to consider other field-relevant measures of toxic effects in freshwater streams, and to seek better biological tools to detect, diagnose and ideally predict community level ecotoxicological impairment. Ecological principles need to be integrated into ecotoxicological research if there is to be a full understanding of the effects of contaminants in real environmental situations (Clements and Rohr, 2009). Luoma and Rainbow (2008, 2010), recognising these challenges, advocated the use of risk assessment approaches with a stronger integration of field observations into decision making processes. Such a ‘lateral risk’ assessment and risk management process, encompassing hitherto separate approaches and using several lines of evidence, recognises the strong potential contribution of observational data from nature in such a decision making process (Luoma and Rainbow, 2008).

In the light of this context, this review concentrates on field measures of toxic effects of trace metal-rich sediment in freshwater streams, with less emphasis on laboratory-based toxicity testing approaches.

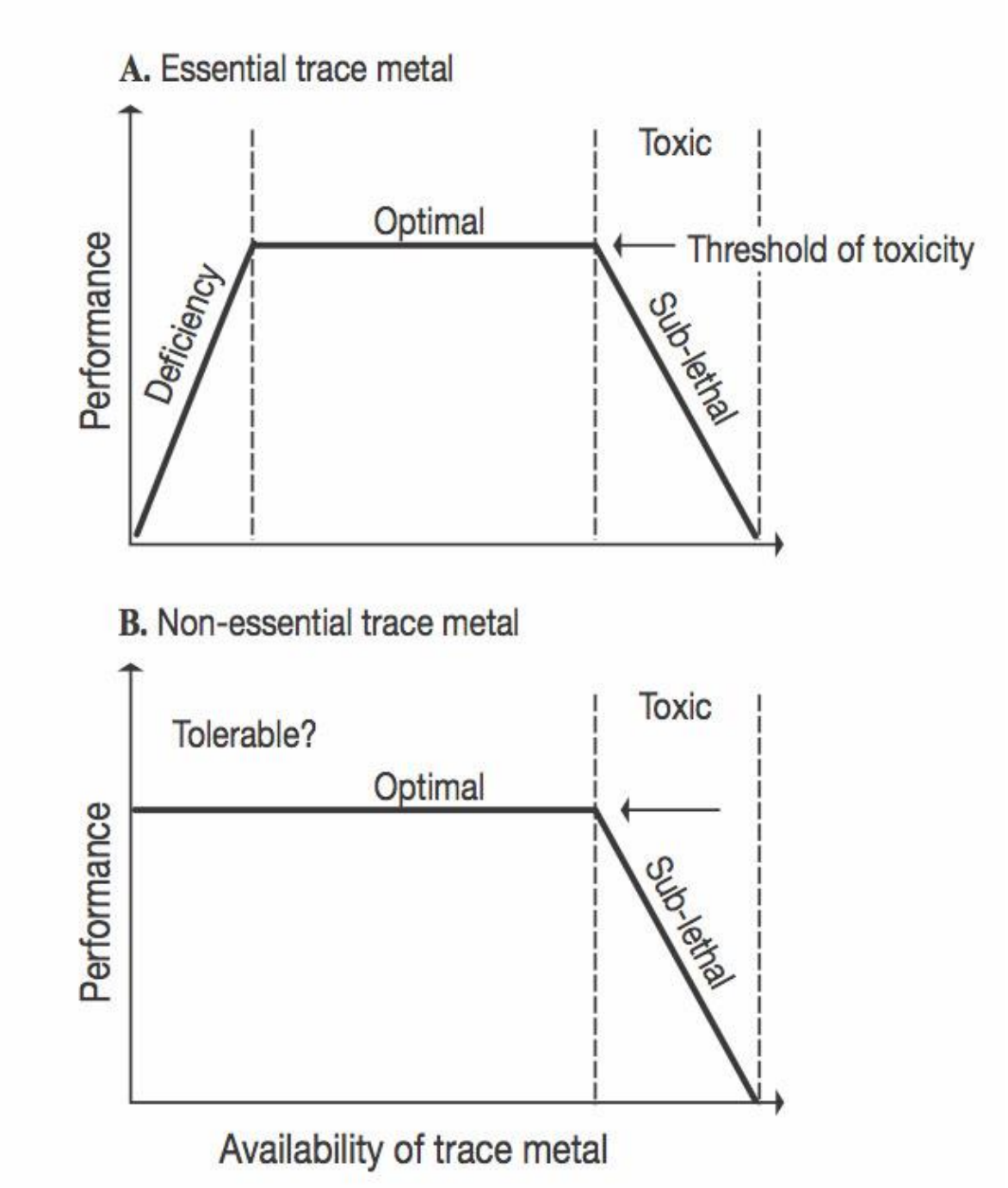


Figure 1.1 Effects of increasing availability of (a) essential and (b) non-essential trace metals on the performance (e.g. growth, production, fecundity, survival, etc.) of an organism (from Luoma and Rainbow, 2008).

1.1.1 General concepts of metal ecotoxicology

Trace metals - those that are typically present in organisms in trace amounts (typically, but not always, less than 0.01% by dry weight in an organism) are most commonly the focus of mining activity. However, definitions of 'trace metals' and other terms e.g. 'heavy metals' (see Luoma and Rainbow, 2008) are inconsistent. For the purposes of this review, we exclude major metals such as sodium, magnesium, potassium and calcium, and also the rare earth elements (lanthanides and actinides). Ores of the latter are mined but are of no relevance in the United Kingdom. Thus the metals of concern potentially include cadmium (Cd), cobalt (Co), copper (Cu), iron (Fe), lead (Pb), manganese (Mn), mercury (Hg), molybdenum (Mo), nickel (Ni), selenium (Se), silver (Ag), tin (Sn), vanadium (V) and zinc (Zn), and the metalloids arsenic (As) and antimony (Sb). These metals may be either the focus of mining activity or present in gangue material and for convenience are all referred to here as trace metals (Luoma and Rainbow, 2008).

An obvious major driver of the detrimental effects of metal-rich sediments derived from mining on the biota of local freshwater streams is the toxicity of the metals themselves. All trace metals, whether essential or non-essential, are toxic to life above a threshold bioavailability (Figure 1.1). This threshold bioavailability may be extremely low or even absent in the case of non-essential metals (Figure 1.2), but it is likely that environmentally low bioavailabilities of a non-essential metal are tolerable without significant sub-lethal physiological cost to an organism (Figure 1.1). In the case of essential trace metals, a very low metal bioavailability will cause negative performance effects as a result of deficiency whilst toxic effects will occur at bioavailabilities above the toxic threshold (Figures 1.1, 1.2). Once toxic effects are apparent (initially sub-lethal and ultimately lethal), these effects will increase in a dose related manner for both essential and non-essential metals (Figure 1.2). It does not follow that non-essential metals are generally more toxic than essential metals, copper being both essential in small doses and yet one of the most toxic metals at higher doses. Nevertheless, it is true that the non-essential metals mercury and silver do top most relative toxicity tables of trace metals (Luoma and Rainbow, 2008).

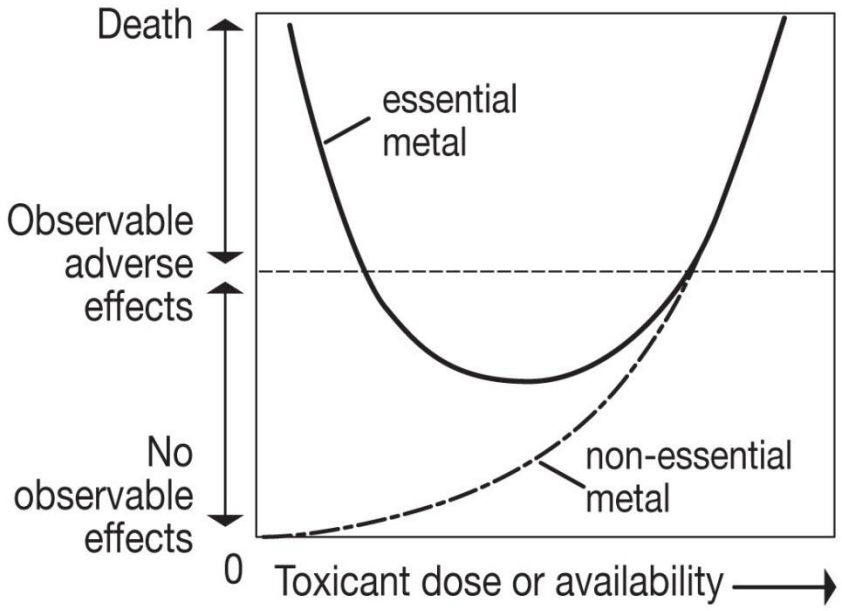


Figure 1.2 Biological response of an organism to increasing dose or availability of an essential or non-essential trace metal (from Luoma and Rainbow, 2008).

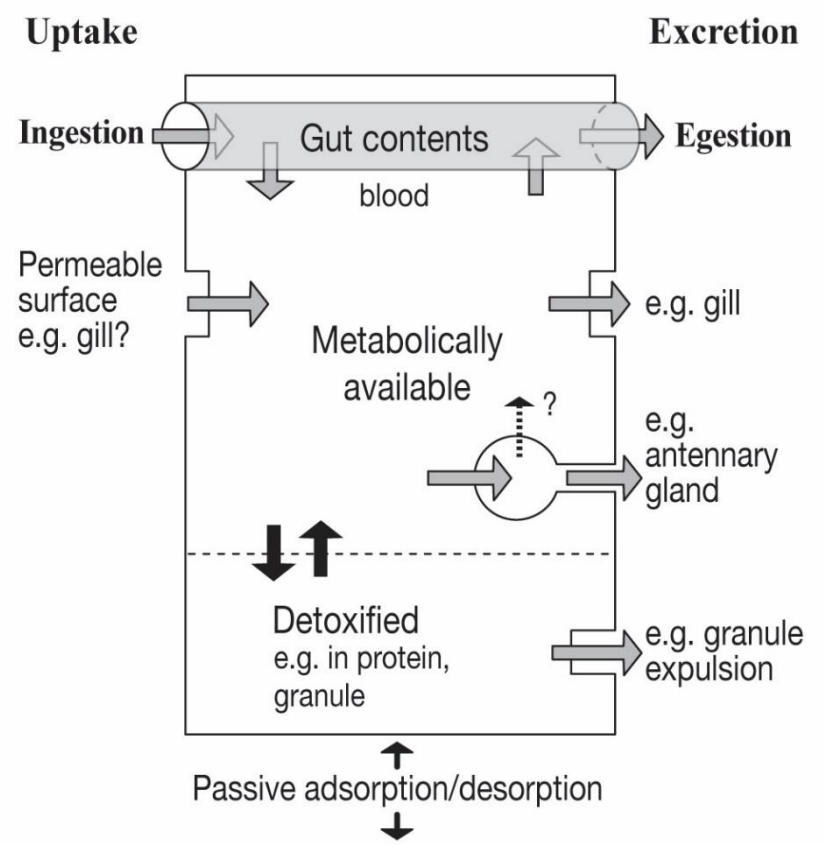


Figure 1.3 A schematic representation of the body metal content of an aquatic invertebrate such as a decapod crustacean. When metal first enters the body, it will initially be metabolically available, before potentially being stored in detoxified form, probably elsewhere in the body after internal transport via body fluids. Detoxified storage may be permanent or temporary. Trace metals taken up into the body may or may not be excreted, either from the metabolically available component or from the detoxified store (from Luoma and Rainbow, 2008).

Trace metals are toxic as a result of their chemical properties, especially their affinity for (preference for binding to) the elements sulphur (S) and nitrogen (N). Sulphur and nitrogen are present in the make-up of many biological macromolecules, especially in proteins, vital to metabolism not least in their guise as enzymes. This affinity for sulphur and nitrogen actually underlies many of the essential roles of trace metals in metabolism, their controlled presence in a protein structure enabling them to act as a catalytic centre in an enzyme or to bind oxygen, as in the respiratory proteins haemoglobin (Fe) and haemocyanin (Cu). The down side of the affinity of trace metals for S and N is that trace metals present in excess, unless prevented from doing so, will inevitably to bind to S- and N-containing groups in biological molecules in the wrong place at the wrong time. They might replace another trace metal playing a key essential role in a protein, thereby inhibiting the catalytic activity of an enzyme, or they might bind elsewhere on the protein, distorting its structure and preventing its biochemical function. Either way a toxic effect has been caused.

Life has evolved in the presence of trace metals and, as indicated above, many of these metals have become incorporated into biochemistry as essential metals playing key metabolic roles. Correspondingly organisms have evolved physiological mechanisms to prevent a build-up of all potentially toxic metals in cells in a metabolically available form that gives them uncontrolled access to bind to the wrong molecule. In practice, trace metals, essential and non-essential, are typically bound in cells to selected molecules that hold them

out of harm's way – either irreversibly or perhaps reversibly in the case of essential metals needed later to fulfil an essential role. These metals have been detoxified by binding to sacrificial sites with a high affinity for that metal, perhaps a special protein (e.g. metallothionein) or to an inorganic granule such as an insoluble metal pyrophosphate. Metals newly entering an organism, for example an aquatic invertebrate, via permeable external surfaces or within the gut are initially in metabolically available form with the potential to bind anywhere. They are usually excreted quickly or detoxified to be removed from harm's way (Figure 1.3).

Toxicity occurs when the total rate of metal uptake combined across all metal uptake routes into an organism exceeds the combined rates of excretion and physiological detoxification (Luoma and Rainbow, 2008). Uptake rates are typically not under the control of the organism and increase passively with the local bioavailabilities of the metals in the local environment. Under most circumstances the combined rate of metal uptake across all metal sources is easily matched by the rate of excretion of the metal, the rate of metal detoxification or by a combination of the two. Under extremely high local metal bioavailabilities toxicity ensues. At and above the toxic threshold (Figures 1.1 & 1.2), the rate of metal uptake is now greater than the combined rates of excretion and detoxification (Croteau and Luoma, 2008, 2009; Casado-Martinez et al., 2010a). Under these circumstances accumulated metals build up intracellularly in a metabolically available form and these metals bind where they are not wanted, with toxic effects (Figure 1.4). Since the rate of uptake of metal increases with increased local metal bioavailability, and since, above the toxic threshold, toxicity is related to uptake rate, it follows that within the toxic range of exposures, toxicity increases with metal bioavailability (Figure 1.4).

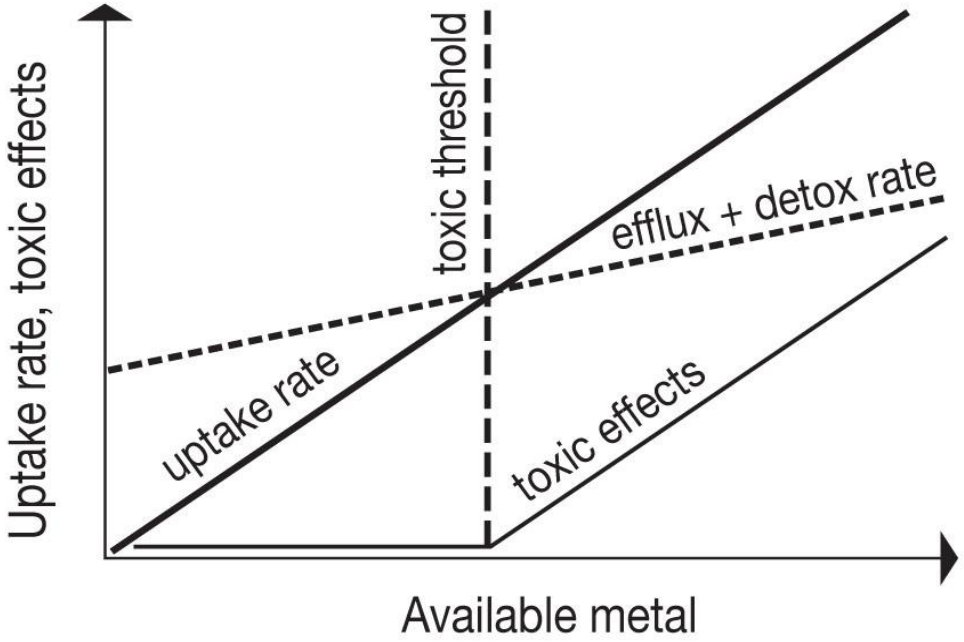


Figure 1.4. Schematic representation of how the uptake rate (combined across all routes of uptake) of a trace metal and hence (after a threshold) the manifestation of toxic effects will increase with the availability of the trace metal to an aquatic organism. Toxic effects occur when the uptake rate exceeds the combined rates of efflux and detoxification (from Luoma and Rainbow, 2008).

1.1.2 Uptake and accumulation of metals in organisms

While the different possible routes of entry of metals across a cell membrane into a cell and, therefore, into an organism are not of specific relevance here (but see Luoma and Rainbow, 2008), it is relevant to consider the different sources of metals available to organisms inhabiting mine-affected fresh water systems. Any organism bathed in water will take up trace metals from solution across the external membrane of cells in contact with the medium. In the case of multicellular animals, a typical route is via the surfaces of respiratory organs, which are particularly permeable, but uptake through other exposed soft tissues does occur. Furthermore, animals will take up metals in the alimentary tract, from ingested material (with availability potentially enhanced after digestion) and water (Croisetière, Hare and Tessier, 2006): the gut wall is specifically adapted for the uptake of materials, making this route especially vulnerable. In the case of sediment dwelling animals, potential routes of metal uptake are direct from solution (typically the water column even if transported into burrows by irrigation currents and, to a lesser or negligible extent, from pore water in the sediment), and from the diet, especially from ingested sediment in the case of deposit feeders. In addition to uptake from solution, sediment-associated herbivores will take up dietary metals from primary producers such as benthic diatoms, macrophytic algae, and/or moss or higher plants associated with sediment. Predators accumulate metals from prey (Croisetière et al., 2006), whether herbivores, deposit feeders or animals at other trophic positions in the food web.

Once metals have been taken up by organisms they may be excreted but are more typically accumulated, a process that necessarily involves the storage of metals in detoxified form (Figure 1.3). If excretion balances uptake, organisms are said to regulate the body concentration of the metal. Regulation of body metal levels is typically restricted to essential trace metals, and is a feature of vertebrates and a few invertebrates, such as decapod crustaceans (Luoma and Rainbow, 2008). Net accumulation patterns vary from weak to strong accumulation, according to the relative rates of metal uptake and excretion, the difference necessarily being made up for by the rate of detoxification. Any accumulator detoxifies the extra metal. If this is stored permanently, then body metal content increases continuously. In some cases, animals can excrete metal in detoxified form, for example as metal-rich granules from the gut or Malpighian tubules. In this case accumulated concentrations may reach steady state levels that vary with local metal uptake rates, and thus local conditions.

Except for those few examples carrying out regulation and therefore for most trace metals in most invertebrates, aquatic invertebrates will have accumulated metal concentrations that vary with local metal bioavailabilities. Toxicity will occur once local bioavailability rises above the toxic threshold. The concentration of metabolically available metal rises before the extra incoming metal can be excreted or detoxified, and this metabolically available fraction can then cause sub-lethal and ultimately lethal toxic effects. The total accumulated metal concentration, however, will depend on the previous history of metal exposure of the invertebrate, and death can occur at any total accumulated metal concentration, depending only on the potentially toxic, much smaller metabolically available concentration. Thus, in contrast with the case of organic contaminants, in any organism that uses detoxification there is no Critical Body Concentration of an accumulated metal at which death occurs – in practice this is true of nearly all invertebrates (Luoma and Rainbow, 2008; Adams et al., 2010).

1.2 Sources, behaviour and consequences of sediment-associated metals in mine-impacted streams

1.2.1 Sources of metals in mine-impacted streams

Metals are produced both in dissolved and particulate form by mineral extraction and a range of ore-processing activities, including smelting, calcination and milling (Mighanetara et al. 2009; Environment Agency 2012a). When metal-bearing sulphide minerals, e.g. pyrite (FeS), galena (PbS) and sphalerite (ZnS), are exposed they are oxidized in the presence of oxygen and water both abiotically and biotically (promoted by Fe-oxidizing *Thiobacillus* bacteria) to release SO_4^{2-}, associated metal cations and colloidal Fe (Singh et al. 1999; Younger et al. 2002; Maia et al. 2012). This often results in a discharge of water which typically has a low pH (pH 2-4), a high ionic strength and a high dissolved metal load, termed 'acid mine drainage' AMD (Butler et al. 2009; Jones et al. 2013), although circum-neutral discharge can also be produced where pH is buffered by the dissolution of carbonate-rich bedrock (e.g. Hiller et al. 2012; Jones et al. 2013). As AMD enters the oxic upland stream environment typical of mining regions, a number of processes occur including a decrease in stream pH, precipitation of Fe oxyhydroxides and the co-precipitation and/or adsorption of dissolved metals to those oxyhydroxides. These precipitates settle out onto the streambed, coat other mineral and sand grains, or can be carried considerable distances downstream in suspension (Hudson-Edwards et al. 1999; Butler et al. 2009; Palumbo-Roe et al. 2012). In addition to these fine-grained, ochreous sediments, mining waste including slag and cinder heaps, tailings ponds, gangue and over-burden can also provide a source of both fine- and coarse-grained metal-rich sediments to the aquatic environment (Macklin et al. 2006; Villarroel et al. 2006; Mighanetara et al. 2009). Consequently, there are numerous point and diffuse sources of metals to the catchment and fluxes of dissolved and particulate metals are highly variable. Seasonal increases in precipitation and groundwater flow and extreme storm/flood events increase the re-suspension of precipitates and the erosion of mining waste materials, as well as the potential collapse of mines and failure of tailings dams (Hudson-Edwards et al. 2003; Mighanetara et al. 2009) releasing large quantities of metal-rich sediment to the catchment. Furthermore, increased flows can result in the remobilization of previously deposited metal rich particulates from the river bed and flood plain.

The geochemistry and physical composition of mine-impacted fluvial sediments can be highly heterogeneous, both spatially and temporally. Streambed sediments comprise detrital mineral grains eroded from mine-waste, e.g. cerussite ($PbCO_3$) and arsenopyrite (FeAsS) (Palumbo-Roe and Colman 2010; Rieuwerts et al. 2014), and Fe-rich sediments precipitated from AMD which, although often described simply as Fe-hydroxides or ferrihydrite, include a wide range of Fe oxyhydroxide, carbonate and hydroxysulphate minerals, including schwertmannite, goethite, jarosite, lepidocrocite and feroxyhite (Singh et al. 1999). In addition, sediments may include efflorescent Fe sulphate precipitates and secondary minerals formed from the oxidation and dissolution/precipitation of detrital mineral grains and Fe-oxyhydroxides, e.g. scorodite ($FeAsO_4.2H_2O$: Hudson-Edwards et al. 1999). Consequently metals can be partitioned to all sediment fractions including carbonates, Fe/Mn oxyhydroxides, sulphides and the residual sediment fractions (e.g. Bird et al. 2003; Byrne et al. 2010; Maia et al. 2012; Wang et al. 2012) with implications for the mobility and bioavailability of metals.

However, the association of metals with the sediment is highly dynamic and is influenced by over-lying water chemistry, hydrological conditions and under-lying geology. For example, changes in stream pH, ionic strength and dissolved organic carbon content can all result in release of metals to the over-lying water column (Butler et al. 2009; Byrne et al. 2010; Palumbo-Roe et al. 2010; Palumbo-Roe and Klinck 2007). Changes in hydrological conditions, for example seasonally, or during storm/flood events, can disturb settled

sediment, changing redox conditions and resulting in the oxidative release of metals (Ranville et al. 2004; Gozzard et al. 2011) and dissolution of efflorescent salts (Mighanetara et al. 2009), as well as the migration of metals to less strongly bound mineral phases. Low flow conditions have also been shown to increase the attenuation of metals, as the over-lying waters can become super-saturated resulting in the precipitation of Fe oxyhydroxides and co-precipitation/adsorption of other metals; conversely, high flow conditions can result in metal release (Byrne et al. 2013a). As a result, metal-rich sediments stored in the catchment in streambed deposits, floodplains and over-bank deposits can present a long-term (100s to 1000s of years) diffuse source of metal contamination to the catchment (Bird et al. 2010; Lecce et al. 2014). These sediment stores can be eroded, transported, dispersed and re-deposited throughout the catchment. Their deposition is highly variable, dependent upon fluvial processes, resulting in 'hotspots' of contamination (Macklin et al. 2006; Dennis et al. 2009). Hence, representative field sampling can be challenging.

1.2.2 Bioavailability of toxic metals in mining affected streams

The word 'bioavailability' has been used in the section above. But what exactly is metal bioavailability? *Bioavailability* describes a relative measure of that fraction of the total ambient metal that an organism actually takes up when encountering or processing environmental media, summated across all possible sources of metal, including water and food as appropriate (Luoma and Rainbow, 2008). Metals taken up by organisms are handled by the physiological processes of the body, typically to be accumulated by most aquatic invertebrates. Although metals from different routes of uptake in aquatic invertebrates (from solution via permeable external surfaces or from the diet via the alimentary tract) may or may not share the exact same physiological routes in the body leading to excretion or detoxified accumulation, the accumulated concentrations are additive, irrespective of the route into the body.

Metals associated with metal-rich sediments can be bioavailable to the local biota in a variety of ways. Classically, it has been considered that metals in solution offer the most significant source of metals to aquatic invertebrates, including those living in or on sediments. Thus, the pore water of sediments has been highlighted (not least by environmental regulators) as a very important source of metals to infaunal animals.
The basic assumption has been that the total concentration of metal in sediment does not matter. It was assumed that the variable driving toxicity was the distribution of metal between sediment particles and water, and exposure was limited to pore waters: this is termed the equilibrium partitioning theory of bioavailability (e.g. Di Toro et al., 1991). In practice, the last decade has brought about a paradigm change in our understanding of the significance of the diet in providing an ecotoxicologically significant (often the major) source of metal to aquatic invertebrates (Wang, 2002; Luoma and Rainbow, 2008). Thus, it has been shown that diet is by far the most important exposure route for cadmium and copper uptake in 5 species of grazing mayfly larvae (Cain et al., 2011). For the predatory alderfly *Sialis velata*, food (prey) is the almost exclusive source of arsenic, cadmium, cobalt, copper, and zinc and the source of 94% of its lead (Croisetière et al., 2006).

Furthermore, it has become increasingly appreciated (Luoma and Rainbow, 2008) that burrowing aquatic invertebrates (including soft bodied worms with potentially permeable body walls) are not actually in contact with undisturbed pore water containing dissolved metal in equilibrium with adjacent metal-rich particles. Burrowing animals live in burrows which are irrigated by a flow of oxygenated water from the overlying water column, so that they can breathe. The contribution of pore water to such bathing water in the burrow is usually negligible, and pore water can, in essence, be considered insignificant as a source of metals to the burrowing animals. Thus, even burrowers are only exposed to dissolved metals from the water column lying above the sediment.

Calculations of how much metal is released from sediment particles into the pore water, and the effects of changes in redox conditions in the pore water, or the sediment content of chemical species such as acid volatile sulphides (AVS) that might bind these metals, are to all extents and purposes irrelevant when considering sources of metals to burrowing invertebrates (Luoma and Rainbow, 2008). It is true that in anoxic sediments one of the most important factors controlling the transfer of metals from sediment to pore water is the presence of acid volatile sulphides (AVS) in the sediment, operationally defined as the amount of sulphides volatilized by the addition of 1N HCl and mainly consisting of iron, manganese and aluminium sulphides (Di Toro et al., 1992). Sulphides bind strongly with metals and inhibit metal exchange from sediment to the pore water, resulting in greatly reduced metal concentrations in the pore water in the zone of anoxia. When such sediments are homogenized and used in toxicity tests, the reduced pore water concentrations correspond to declines in toxicity (Ankley et al., 1996). In 10 day whole sediment toxicity tests, acute toxicity is typically not observed when the molar concentration of AVS is greater than the molar concentration of the simultaneously extracted metals (SEM) released from a sediment sample during AVS extraction (Di Toro et al., 1991). The body of literature relating sediment toxicity to SEM-AVS in whole sediment bioassays is large, but the conceptual basis of the SEM-AVS approach is flawed because burrowing animals live in oxidised subsections (burrows) of otherwise anoxic sediments and are not bathed in anoxic pore water. Toxicity tests with homogenised sediments break down this substructure and have no relevance to field conditions. Despite these constraints, the whole sediment bioassay paradigm has been repeatedly used (Ankley et al., 1996), and equilibrium partitioning was assumed to be the basis of the toxicity of sediment-associated metals to burrowing animals (Di Toro et al., 1992).

De Jonge et al. (2009) evaluated the influence of AVS on accumulation of sediment-bound metals in sediment burrowing invertebrates (midge larvae *Chironomus* gr. *thummi* and oligochaete worms *Tubifex tubifex*) under field conditions (17 historically polluted Flemish rivers). They showed that AVS was not a significant variable in describing variation in metal accumulation, and clearly demonstrated that burrowing invertebrates can accumulate metals from field sediments even when there is an excess of AVS present in the sediments. Supporting evidence comes from much related literature (Hare et al., 1994; Ingersoll et al., 1994; Lee et al., 2000a, b; Yoo et al., 2004). Similarly, De Jonge et al. (2010) evaluated the relationship between AVS and metal accumulation in the two above species and a further four freshwater species; they concluded that when uptake through food becomes the dominant metal exposure route, metal accumulation in an organism is not controlled by AVS concentrations in the sediment.

The diet is a, if not the, major source of trace metals to aquatic invertebrates (Wang, 2002; Luoma and Rainbow, 2008; De Forest and Meyer, 2015). Supporting evidence is provided from the results of a modelling technique that is relatively new – biodynamic or biokinetic modelling (Wang et al., 1996; Luoma and Rainbow, 2005). This predominance of diet as a metal source is even more exaggerated for deposit feeders which ingest large amounts of fine-grained sediment with a high surface area to volume ratio and typically high organic content, both attributes promoting the adsorption of high concentrations of trace metals. Biodynamic modelling takes into account such high ingestion rates as well as the assimilation efficiency of the ingesting animal, together with the high metal concentrations in sediments, to show that the sediment ingestion route often provides in excess of 90% of total metal uptake in aquatic deposit feeders (Casado-Martinez et al., 2009, 2010b; Rainbow et al., 2009).

Nevertheless, we still suffer from the hangover of acute dissolved toxicity tests in setting Environmental Quality Standards, on the long-held assumption that dissolved metal represents the most significant source of metals to aquatic invertebrates. Whilst this is true in the artificial experimental set-up used for acute laboratory toxicity testing, it is a false

premise when attempting to predict the onset of toxicity in field situations. The paradigm needs to continue to change as more ecology is introduced into ecotoxicology (Clements and Rohr, 2009; Luoma and Rainbow, 2010).

1.2.3 Biomonitors

Most aquatic invertebrates are accumulators of trace metals, storing most of the metal taken up in detoxified form (Luoma and Rainbow, 2008; Rainbow and Luoma, 2011a). Strong accumulators may excrete very little of the metal accumulated, whereas weaker accumulators may lose some metal from the body, perhaps still in detoxified form (Figure 1.5: Luoma and Rainbow, 2008). In the latter case there will be a turnover of metal but the body concentration will still reflect local bioavailabilities, the standing stock of metal in the body during turnover being higher when bioavailability is high, as depicted in Figure 1.6 (Luoma and Rainbow, 2008). It is possible to estimate the rate of turnover of the accumulated metal in the body using biodynamic modelling, and thus, estimate the period of exposure reflected in that accumulated body metal content. This may be of the order of days, weeks, months or years depending upon the metal and the invertebrate species concerned. Any organism that is a metal accumulator has the potential to act as a biomonitor.

A *biomonitor* is defined here as an organism which accumulates trace metals in its tissues, the accumulated metal concentration of which provides a relative measure of the total amount of metal taken up by all routes by that organism, integrated over a preceding time period (Luoma and Rainbow, 2008). Thus, the bioaccumulated concentrations are integrated measures of the uptake and accumulation of metals from all sources to that organism. The use of biomonitors can, therefore, identify areas of high or low metal bioavailability to that chosen biomonitor, and to identify changes of metal bioavailability in space and/or time. Such information is prerequisite to an efficient subsequent search for (sub-lethal) ecotoxicological effects.

This use of the term biomonitoring is more restricted here than might be the case for some authors. For example, Clements and Newman (2002) use the term *biomonitoring* synonymously with *biological monitoring* (or *bioassessment*) to mean the use of biological systems to assess the structural and functional integrity of aquatic and terrestrial ecosystems. We use the term biological monitoring, but not the term biomonitoring, to cover the wider context of the use of any biological system in ecosystem assessment. While we do consider biomonitoring as defined here to be a subdivision of biological monitoring, we do not go as far as Roux et al. (1993) who used biomonitoring in the same wider context of Clements and Newman (2002), but subdivided it as follows: *Bioassessments* are based on ecological surveys of the functional and/or structural aspects of biological communities. *Toxicity bioassays* are a laboratory-based methodology for investigating and predicting the effect of compounds on test organisms. *Behavioural bioassays* explore sub-lethal effects of species when exposed to contaminated water; usually as on-site, early warning systems. *Bioaccumulation* studies monitor the uptake and retention of chemicals in the body of an organism and the consequent effects higher up the food chain. There is little value in pursuing the terminology argument here, but it is necessary for us to be clear as to how we are using the term biomonitoring, which will appear often in this review.

Strong accumulators typically show a wider range of accumulated concentrations than weak accumulators over a bioavailability range, and hence offer greater discriminatory ability between sites or between sampling occasions at the same site. Nevertheless, weak accumulators can still be used as biomonitors, often introducing the advantage of reflecting a shorter recent period of metal exposure. While conclusions on local metal bioavailabilities are often drawn from data for single biomonitors, strictly those results only apply to the integrated metal sources available to that biomonitor, and a biomonitoring programme

should typically employ a suite of biomonitors to cover a wider range of potential metal sources in a habitat. If a suite of biomonitors is used, comprising a selected group of biomonitors that take up and accumulate metals from a variety of sources (e.g. solution, seston, deposited sediment, prey, etc.), comparative information on the relative importance of different bioavailable sources of metals in a specific habitat can be provided (Luoma and Rainbow, 2008).

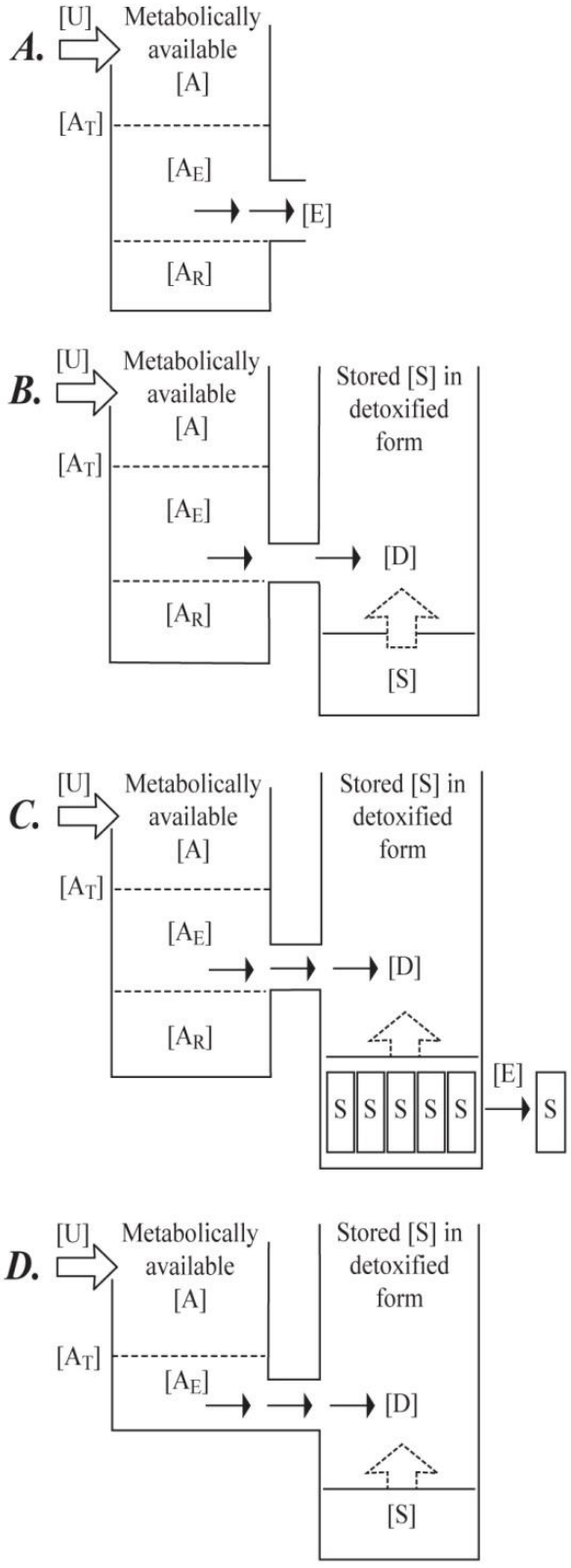


Figure 1.5. Trace metal accumulation patterns of aquatic invertebrates.

(A). The trace metal accumulation pattern of an aquatic invertebrate which regulates the total body metal concentration of an essential metal by balancing uptake [U] with excretion [E]. All metal is accumulated in the metabolically available component [A], itself subdivided into the essential metal required for metabolic purposes [A_R], and excess metal [A_E] over and above this metabolic requirement. There is a threshold concentration [A_T] of metabolically available metal, above which the accumulated metal is toxic.

(B). The trace metal accumulation pattern of an aquatic invertebrate that is a net accumulator of an essential metal without significant excretion of metal taken up. Metabolically available metal in excess of requirements is detoxified [D] to be stored [S] as the detoxified component of accumulated metal with no upper concentration limit.

(C). The trace metal accumulation pattern of an aquatic invertebrate that shows net accumulation of an essential metal in detoxified form, but excretes some of that accumulated metal in the detoxified component. Examples of this accumulation pattern are those of copper and zinc accumulated from the diet by amphipod crustaceans (see Figure 1.6).

(D). The trace metal accumulation pattern of an aquatic invertebrate that shows net accumulation of a non-essential metal in detoxified form with no significant excretion. (After Luoma and Rainbow, 2008)

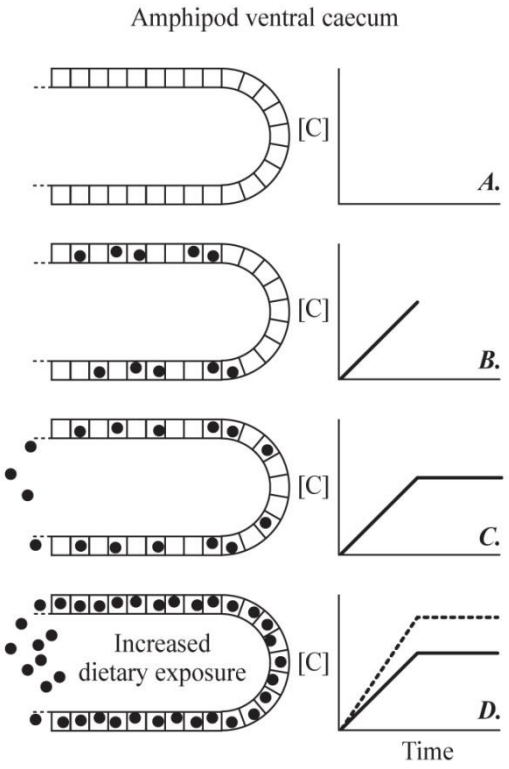


Figure 1.6. The accumulation of detoxified metal (e.g. zinc or copper) in the ventral caecal cells of an amphipod crustacean exposed to a trace metal in the diet, and the corresponding changes in total body metal concentration [C] over time. D. The effect of increased dietary exposure to the metal. (After Luoma and Rainbow, 2008)

1.2.4 Ecotoxicological effects – Biomarkers

While biomonitors as defined above will provide information on when, where and how much of a metal in bioavailable form(s) is present in a habitat, biomonitoring does not directly address the question 'So what?' Biomonitoring does have the great preliminary advantage of informing us where and when to look for likely ecotoxicological effects of the metals under consideration. To answer the 'So what?' question is to ask whether an observed high bioavailability of a metal is actually having an ecotoxicological effect on a member of the local biota, and to question the severity (ecotoxicological significance) of that effect. For the answers to such questions we turn to biomarkers.

A *biomarker* is a biological response (e.g. a biochemical, cellular, physiological or behavioural variation) that can be measured at the lower levels of biological organization, in tissue or body fluids or at the level of the whole organism. In short a biomarker is a measurable biological response to the local presence of a bioavailable quantity of a contaminant (a toxic metal in this context).

Ecotoxicological effects occur at different levels of biological organisation – through the molecular, biochemical, cellular, physiological, individual organism, population, and community levels (Figure 1.7). Any biological response will be first elicited at the lowest level of biological organisation. Thus, a single metal ion taken up may bind to a single protein molecule. A singular event like this has no toxic significance, but with increased metal uptake, more and more metal will bind to intracellular molecules with potential toxic effects. The molecular response is the most sensitive, followed in turn by effects progressing up the hierarchy of biological organisation. While biomarkers at the lowest levels of biological organisation are the most sensitive (Figure 1.8), it is the toxicological effects reflected by biomarkers at the highest level (the community) that are most ecotoxicologically significant. If metal pollution (high metal bioavailability) is producing ecotoxicological effects observable at the community level, then biota are being greatly affected. The task facing environmental

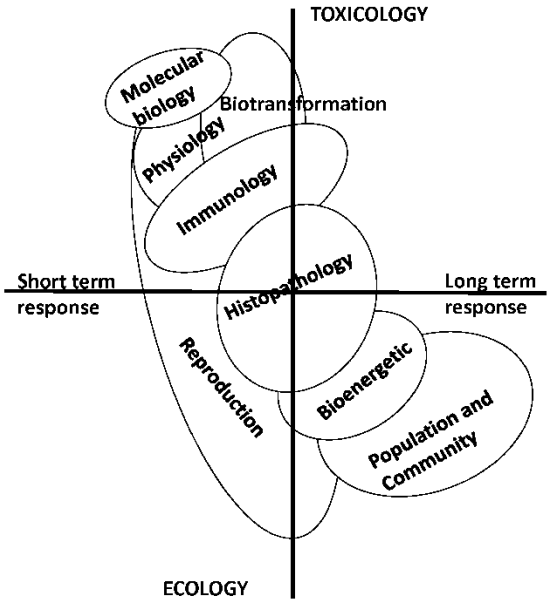


Figure 1.7. Biomarkers: latency between exposure of fish to pollutants and the occurrence of biological effects at different levels of biological organisation (from Amiard-Triquet and Amiard (2013a) after Adams et al., 1989).

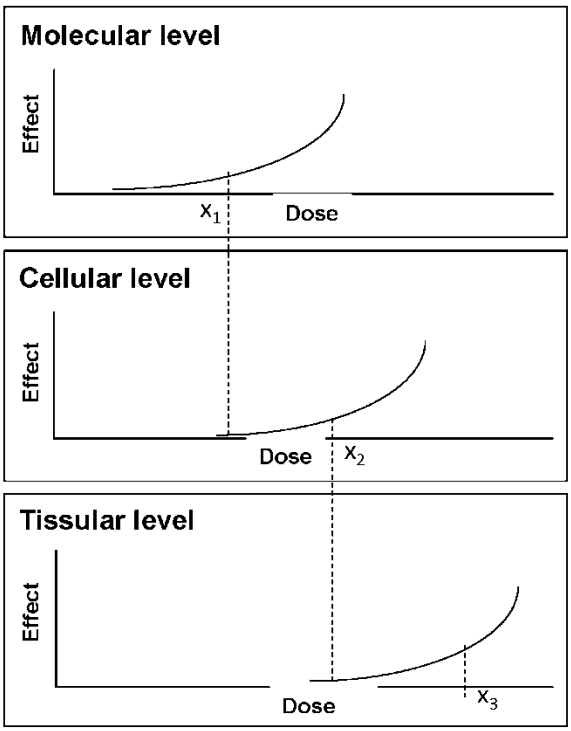


Figure 1.8. Biomarkers: progression of the dose-effect relationship from low to higher levels of biological organisation (from Amiard-Triquet and Amiard, 2013a).

regulators is to detect where the ecotoxicological effects of metal contamination are causing profound damage to biota.

A key objective in recent years has been to detect biomarkers at lower levels of biological organisation that can be shown to correlate with potential effects at higher levels of organisation. Much progress has been made recently. Moore and colleagues (e.g. Moore et al., 2013) have correlated an easily measurable lower level cellular biomarker, lysosomal stability (for details see below) in mussels to higher order physiological measures of Total Oxyradical Scavenging Capacity (a biomarker of antioxidant defence activity), and to scope for growth (the energy available to an organism for growth and reproduction after the physiological cost of resisting the ecotoxicity of a contaminant: Figure 1.9). Thus, we can now measure a lower level biomarker with more confidence that the measurement has ecotoxicological relevance. We suggest that a toolkit of biomarkers at various level of organisation should be used to detect whether a contaminant is really having a significant ecotoxicological effect in a metal-contaminated habitat (Amiard-Triquet, 2015).

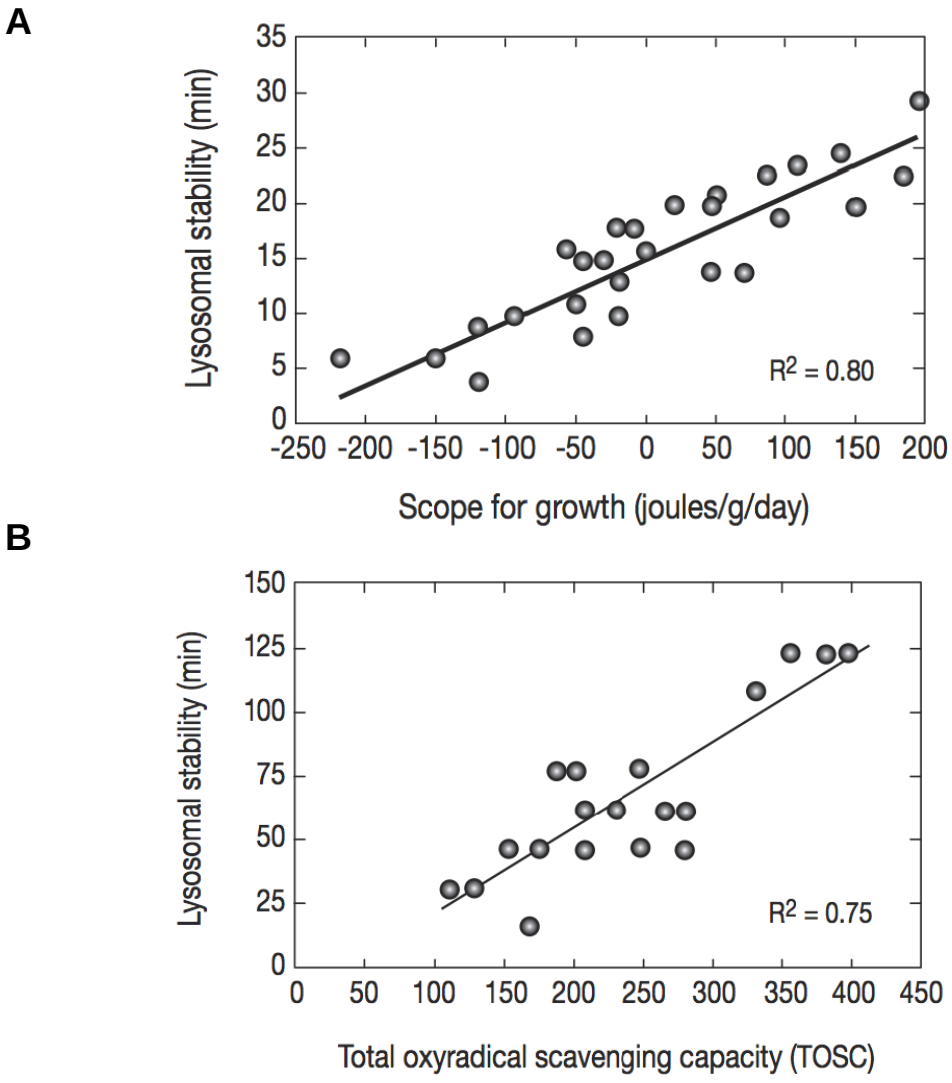


Figure 1.9. Significant correlations of biomarkers from low to higher levels of biological organisation. A. Between scope for growth and lysosomal stability in the mussel *Mytilus edulis* (Allen and Moore, 2004). B. Between lysosomal stability and Total Oxyradical Scavenging Capacity (TOSC) in mussels (after Regoli, 2000; Moore et al., 2006). (From Luoma and Rainbow, 2008)

1.2.5 Current water management legislation

The European Union has harmonized the management of water quality across member nations through the application of the Water Framework Directive (WFD) which was passed in 2000 (European Parliament, 2000). The WFD commits member states to achieving good qualitative and quantitative status of all water bodies, including rivers, lakes, estuaries, coastal waters and groundwater. Both ecological status and chemical status need to be at least good to achieve this *Good Water Status*. The WFD uses ambient environmental standards to classify water bodies on the basis of chemical contamination, and these standards are set to prevent ecological damage from contaminants like trace metals. A particular focus is given to establishing maximum acceptable concentrations of 'priority hazardous substances' which present a particularly strong environmental risk (European Parliament and European Council, 2001, 2006); the measurement of these substances in surface waters constitutes a major tool for regulatory agencies in their efforts to achieve the requirements of the WFD. The environmental standards, however, are only one of several benchmarks. Success is judged by the achievement of ecological goals, rather than by meeting purely chemical standards. Thus, the WFD places a legal obligation on EU nations to use biota to assess the ecological quality of a water body (Jones et al.; 2010). The ecological objectives are designed to protect and, where necessary, restore the structure and function of aquatic ecosystems, and thereby safeguard the sustainable use of water resources.

The Water Framework Directive requires that the current status of water bodies must be determined using a formal classification scheme that characterizes the ecological status of each water body based on biological quality elements, and the hydromorphological, chemical and physico-chemical elements that support the biological elements. The Directive identifies a list of priority hazardous substances, which includes many metals, which should be managed to achieve concentrations in the environment near background values. Where sites are failing to achieve good or better ecological status, measures must also be identified that will achieve and maintain the objective of good status. The WFD requires on-going monitoring to assure achievement of these objectives. Here, the ecological status is compared with a reference condition, where reference condition represents the condition of a water body considered to be in a relatively un-impacted state. Such a reference condition provides a calibration point against which the quality of other water bodies can be assessed.

One aspect of the WFD approach is delivered by REACH (Registration, Evaluation and Authorization of Chemicals), a June 2007 regulation (European Parliament and European Council 2007, and updated thereafter) which aims to improve the protection of human and environmental health through better and earlier identification of the toxic properties of chemicals produced by industry. REACH puts responsibility on industry to manage risks from chemicals emitted and to provide safety information on the potential hazard or toxicity of the chemical concerned, and information on amounts emitted. REACH has particular relevance for industrial concerns currently emitting potential hazardous effluent into water bodies, but has limited relevance when considering metal-rich sediments deposited into streams in previous eras of industrial mining activity.

While the recognition by the WFD of the need for the assessment of ecological status of a water body is to be welcomed, Environmental Quality Standards (EQS) are still very significant in assessing the water quality status of a water body in the terms laid down by the WFD. For reasons explained above, not least the significant role of the diet in addition to dissolved metal in providing ecotoxicologically significant doses of toxic metal to local stream biota, it is not easy to translate the toxicity performance of a defined dissolved metal concentration from toxicological action in the laboratory to ecotoxicological action in the field. Nevertheless, Environmental Quality Standards for trace metals in the aquatic environment have been defined for the UK and other member states (Environment Agency, 2008a: Table

1.1), and the dissolved metal QS proposed under the WFD are similar to the metal concentrations in rivers associated with unimpaired benthic invertebrate assemblages in England and Wales (Crane et al., 2007). Similar environmental standards have been published for North America and Australia (Table 1.1): yet such standards are rarely defined for the metal content of sediments. Streams with metal-rich sediments derived from mining, but deposited long ago, may have water low in dissolved metal flowing over these sediments; it may be the case then that dissolved metal EQS alone may have little ecotoxicological relevance.

As the WFD requires member states to achieve 'Good' ecological status, biological techniques are now urgently required that can predict the consequences and cost-effectiveness of water body management measures (Jones et al., 2010). Thus we need more biological monitoring tools to predict and support the return of 'Good' ecological status to contaminated water bodies. Currently, in requiring biota to be used to assess the ecological quality of a water body, the WFD focuses on community-level biological impacts on the biological quality elements (comprising macroinvertebrates, phytobenthos (aquatic plants and benthic algae), phytoplankton and fish depending on the water body type), although a water body will still fail to be classified as 'Good' if the average concentration of any metal listed as a priority substance exceeds the relevant environmental quality standard for that specific metal. This review will consider community level biological methods to assess the ecological status of streams with metal-rich sediments, but it will also make use of the established correlations between biomarkers at low and high levels of biological organisation (Amiard-Triquet et al., 2013b) to assess the predictive applicability of new biological tools.

1.3 Environmental assessment of mining-affected streams

1.3.1 Mining-affected river systems – a UK perspective

The Environment Agency has funded previous projects of relevance to this review, such as Science Project SC030136 on Abandoned Mines and the Water Environment (Environment Agency, 2008b), including an Assessment of Metal Mining-Contaminated River Sediments in England and Wales (SC030136/SR4: Environment Agency, 2008c), and the Nature of Waste Associated with Closed Mines in England and Wales (Palumbo-Roe and Colman 2010). Of particular specific relevance is Report SC030136/R49 (Environment Agency, 2009), a review of the literature on Ecological Indicators for Abandoned Mines. These reports offer a starting point for this review. This review will explore new ground on more recent developments on field measures assessing the toxic effects of metal-rich sediments in streams.

A further Environment Agency Science Report SC030136/R2 (Environment Agency, 2012) addresses the Prioritisation of Abandoned Non-Coal Mine Impacts on the Environment and provides additional data on discharges from mine sites.

Metal ores have been mined in Britain for more than 4,000 years with nearly 5,000 mine sites identified (Environment Agency, 2008b, c, 2012), although currently very few metal mines are still in use. A new tungsten and tin mine opened at Hemerdon, Devon, in 2015: there are also moves to reopen the South Crofty tin mine in Cornwall, which closed in 1998, and zinc exploration work being undertaken in the North Pennine field around Nenthead and Allenheads. Historically, hundreds of thousands of tonnes of lead, zinc and copper ore have been extracted from major mining regions such as the Northern Pennines and Yorkshire Dales, North and Central Wales, the Isle of Man, Derbyshire, and Cornwall and Devon, while many other trace metals including iron, tin, arsenic and silver have also been mined (Environment Agency, 2008c). Past mining methods involved the use of water and significant quantities of metal-rich, fine-grained sediments were transferred into local river systems.

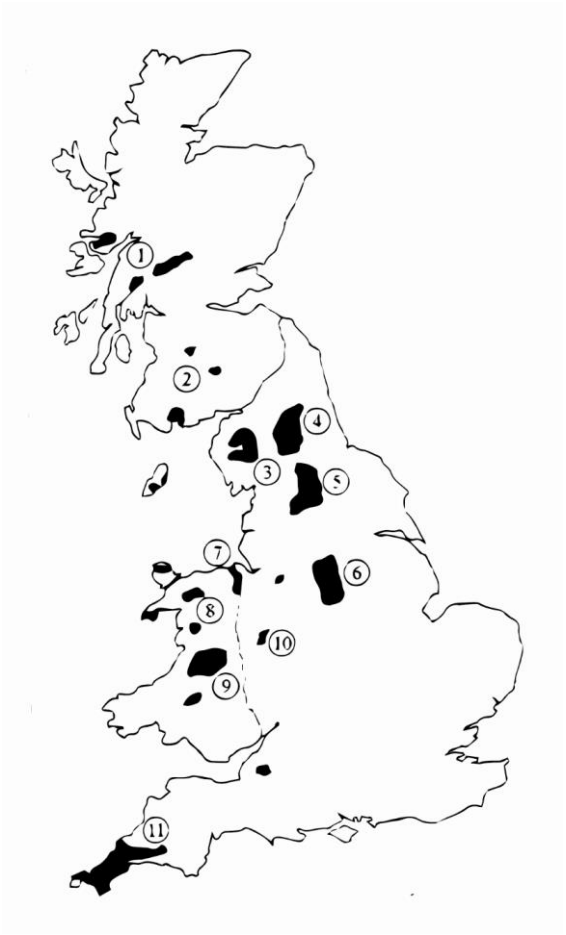


Figure 1.10. Principal metalliferous ore fields. 1. West Scotland. 2. Southern Uplands. 3. Lake District. 4. Northern Pennines. 5. Yorkshire Dales. 6. Peak District/Derbyshire. 7. North Wales – Halkyn Minera. 8. Snowdonia - Llanrwst Harlech, Parys Mountain. 9. Central Wales. 10. West Shropshire. 11. Devon-Cornwall. (from Downing et al. 1998)

Dissolved metals are typically derived from the oxidation of metal sulphides like galena (PbS), sphalerite (ZnS) and greenockite (CdS), leading to raised dissolved metal concentrations downstream of mining sites (Environment Agency, 2009). Pyrite (iron sulphide) may (but not always) be present in association with such other metal sulphides, leading to raised dissolved iron concentrations and the visible deposition of iron (oxy)hydroxides (ochre) further downstream (Environment Agency, 2009). The precipitation of ochre blanketing the stream bed is a particular feature of the iron-rich discharges from abandoned coal mines.

While currently very few mines are actively worked in Britain, some abandoned metal mines do still contribute significantly to the trace metal pollution entering British rivers (Environment Agency, 2008b: Figure 1.11). The Parys Mountain copper mine on Anglesey discharges 24 tonnes of zinc and 10 tonnes of copper every year into the Irish Sea via the Afon Goch and its estuary Dulas Bay; Restronguet Creek in Cornwall, fed by the River Carnon, discharges 52 tonnes of zinc, 12 tonnes of copper and 62 kg of cadmium annually; the combined effect of 50 abandoned metal mines in Wales is an annual discharge of 200 tonnes of zinc, 32 tonnes of copper, 15 tonnes of lead and 600 kg of cadmium (Environment Agency, 2008b). Although cadmium is not specifically sought by the relevant mines, its presence in mined ores does represent an ecotoxicological threat downstream, particularly since cadmium is a priority hazardous substance and more than 70% of failures to achieve its freshwater quality

standard are in mining areas (Environment Agency, 2008b). The English and Welsh river catchments at risk or probably at risk of mining-related metal contamination are illustrated in Figure 1.11 and listed in Table 1.2 (from Environment Agency, 2008c), the major metals of concern being lead, zinc, copper and cadmium.

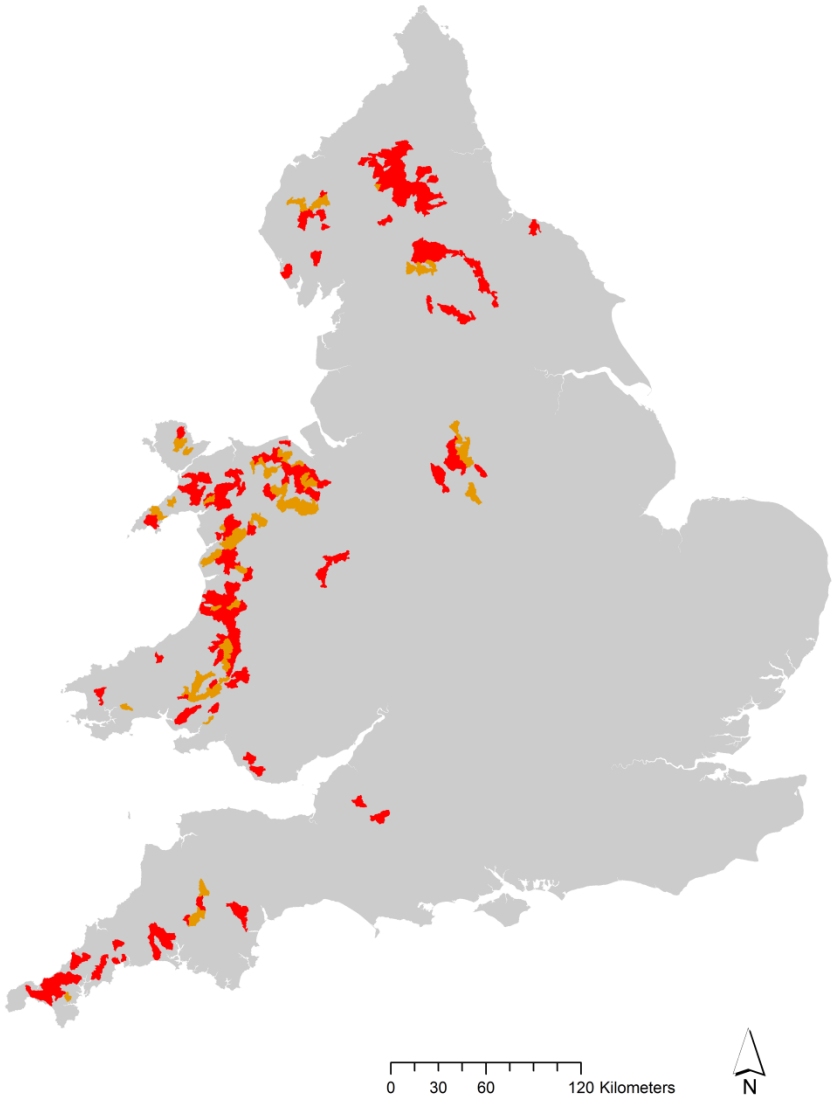


Figure 1.11. Water bodies at risk (red) or probably at risk (orange) of impacts from metal mining.

Table 1.1a. Freshwater Environmental Standards for Metals and Metalloids (in µg l^{-1})

		UK EQS[1,2,3]		Australia & NZ 95% trigger[4]	USA[5]		Canada[6]	
	Hardness (mg $CaCO_3$ l^{-1})	Long-term (Mean)	Short-term (95 %ile)	Annual Average	Chronic CCC	Acute CMC	Long-term	Short-term
Aluminium				55*			5*	
Arsenic		50			150	340	5	
Arsenic III				24				
Arsenic V				13				
Boron				370			29,000	1,500
Cadmium	< 40	≤ 0.08	≤ 0.45	0.2				
	40–50	0.08	0.45				0.09	1
	50–100	0.09	0.6					
	100–200	0.15	0.9		0.25	2		
	>200	0.25	1.5					
Chromium III		4.7	32		74	570	1.0	
Chromium IV		3.4		1	11	16	8.9	
Cobalt		3†	100†					
Copper	0–50	1 bioavailable		1.4			2	
							2-4°	
							2-4°	
							4	
Iron		1000			1000		300	
Lead		7.2		3.4	2.5	65	1°	
Manganese		123 bioavailable		1900				
Mercury		0.05	0.07	0.6	0.77	1.4	0.026	
Molybdenum								73
Nickel		20		11	52	470	25°	
Selenium				11			1	
Silver		0.05†	0.1†	0.05		3.2	0.1	
Tin		25†						
Vanadium	0-200	20‡						
	>200	60‡						
Zinc		10.9 bioavailable + Ambient Background Concentration						
	0–50			8			30	
	100–250				120	120		

Table 1.1b. Saltwater Environmental Standards for Metals and Metalloids (in µg l^{-1})

	UK EQS [1,2,3]		Australia & NZ 95% trigger[4]	USA[5]		Canada[6]
	Long-term (Mean)	Short- term (95 percentile)	Annual Average	Chronic CCC	Acute CMC	Long-term
Arsenic	25			36	69	12.5
Cadmium	0.2		5.5	8.8	40	0.12
Chromium III			27.4			1.5
Chromium IV	0.6	32	4.4	50	1100	56
Cobalt	3†	100†	1			
Copper	3.76 where DOC ≤1mg/l; 3.76 + (2.677 x ((DOC/2) – 0.5)), where DOC >1mg/l		1.3	3.1	4.8	
Iron	1000					
Lead	7.2		4.4	8.1	210	
Mercury	0.05	0.07	0.4	0.94	1.8	0.016
Nickel	20		70	8.2	74	
Silver	0.5	1	1.4		1.9	
Tin	10†					
Vanadium	100‡		100			
Zinc	6.8 dissolved + Ambient Background Concentration		15	81	90	

* pH<6.5 Australia & New Zealand, pH <5 Canada (100 µg l^{-1} for pH >5)
†Non-statutory. Dangerous Substances Directive (76/464/EEC)
‡Statutory. Dangerous Substances Directive (76/464/EEC : European Council 1976)
http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:31976L0464
° variable dependent on measured hardness.
[1] WFD-UKTAG (2008a) Proposals for Environmental Quality Standards for Annex VIII Substances Final (Revised)
[2] DEFRA and Welsh Government (2014) Water Framework Directive implementation in England and Wales: new and updated standards to protect the water environment. https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/307788/river-basin-planning-standards.pdf
[3] European Commission (2012) Directive of the European Parliament and of the Council amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy. COM (2011) 876 final
[4] ANZECC and ARMCANZ [Australian and New Zealand Environment and Conservation Council, Agriculture and Resource Management Council of Australia and New Zealand] (2000) *Australian and New Zealand guidelines for fresh and marine water quality. Volume 1, The guidelines.* National water quality management strategy; no.4
[5] US EPA National Recommended Water Quality Criteria - Aquatic Life Criteria Table http://water.epa.gov/scitech/swguidance/standards/criteria/current/index.cfm
[6] Canadian Council of Ministers of the Environment. 1999. Canadian water quality guidelines for the protection of aquatic life. Canadian Council of Ministers of the Environment, Winnipeg. http://ceqg-rcqe.ccme.ca/

Table 1.1c. Freshwater Sediment Quality Guidelines (in mg/kg dry wt.)

	Australia and New Zealand*		Belgium (Flanders)†	Canada°	
	Low Trigger Value (10% probability of effect)	High Trigger Value (50% probability of effect)	SQG	Interim sediment quality guidelines	probable effect level
Cadmium	1.5	10	1	0.6	3.5
Chromium	80	370	62	37.3	90
Copper	65	270	20	35.7	197
Lead	50	220	40	35	91.3
Mercury	0.15	1	0.55	0.17	0.486
Nickel	21	52	16		
Silver	1	3.7			
Zinc	200	410	147	123	315
Antimony	2	25			
Arsenic	20	70	19	5.9	17

* ANZECC and ARMCANZ [Australian and New Zealand Environment and Conservation Council, Agriculture and Resource Management Council of Australia and New Zealand] (2000) *Australian and New Zealand guidelines for fresh and marine water quality. Volume 1, The guidelines.* National water quality management strategy; no.4
† Flemish sediment quality guidelines as published on July 9th 2010 (de Deckere et al., 2011).
° Canadian Council of Ministers of the Environment. 1999. Canadian sediment quality guidelines for the protection of aquatic life: Winnipeg

Table 1.2. River basins most affected by metal mining in England and Wales. (From Environment Agency, 2008b).

Water Framework Directive River Basin District	Catchment	Mining Region Ore Field
Dee	Clywedog	Halkyn-Minera
Humber	Swale, Wharfe, Nidd, Ure Ecclesbourne, Hamps, Manifold, Derwent	Yorkshire Dales Southern Pennines (Derbyshire)
Northumbria	South Tyne, Wear, Tees	Northern Pennines
North West	Newland's Beck, Coledale Beck	Lake District
Severn	Rea Beck Upper Severn Yeo, Axe	West Shropshire Central Wales Mendip
Solway Tweed	Glenridding Beck	Lake District
South West	Camel, Erme, Fal, Fowey, Gannel, Tamar	Devon-Cornwall
Western Wales	Afon Goch Twymyn, Rheidol, Ystwyth	Parys Mountain Central Wales

1.3.2 Sediment quality

The presence of fine-grained (> 63 μm) sediments and sediment-associated metals in streams and floodplains has the strong potential to cause a significant long-term ecotoxicological impact (Environment Agency, 2008b). In England and Wales the prime metals of concern are copper, lead, zinc and cadmium, with arsenic also being significant in Cornwall and Devon (Environment Agency, 2008b). Table 1.3 provides selected data on sediment metal concentrations in river systems in England and Wales affected by historical mining; these concentrations may exceed geochemical background concentrations and proposed Environment Agency guidelines by several orders of magnitude (Environment Agency, 2008b). The worst affected rivers in terms of sediment metal concentrations are the South Tyne and its tributaries, the Nent, Allen, upper Derwent, the upper Tees, upper Swale and the Ystwyth (Environment Agency, 2008b).

Table 1.3. Selected metal concentrations (μg g^{-1}) in river and overbank sediments affected by metal mines and proposed Environment Agency sediment quality guidelines. (From Environment Agency, 2008b).

River System	Sediment	As	Cd	Cu	Pb	Zn
Swale	fine channel		0–30		22–20,310	26–12,203
Tamar	channel	4–11,000	0.25-22.1	11–8,000	13-450	48–1,901
West Allen (Tyne)	overbank		5-33	22-40	98–3,166	74–1,131
Nent (Tyne)	overbank				224–15,800	4,360–38,000
Tyne & South Tyne	overbank		2.3-117	8-384	410–9,798	590–16,520
Hudeshope Beck	overbank		<0.05-17	0.5-388	63– 26,800	190 – 5,180
Axe	overbank			3 - 27	226 – 25,124	89 - 660
Ystwyth	overbank				73 – 4,646	123 – 1,543
Guidelines - sediments						
Threshold Effect Level (TEL)		**5.9**	**0.596**	**36.7**	**35**	**123**
Predicted Effect Level (PEL)		**17**	**3.53**	**197**	**91.3**	**315**

1.3.3 Ecosystem responses to metal exposure in streams

Mining contamination of streams and rivers produces ecological damage (Environment Agency, 2008b, 2009; Luoma and Rainbow, 2008). While individual case studies may not include all ecological symptoms and the relationship between the onset of negative ecological effects and metal concentrations in water or sediment (the dose response) will vary from site to site, a combination of several signs is diagnostic of the ecotoxicological effects of mining on streams and rivers (Luoma and Rainbow, 2008). These effects include reduced numbers and diversity of aquatic flora and invertebrates (Say and Whitton, 1981a, b; Jarvis and Younger, 2000; Clements, 2004; Environment Agency, 2008b, 2009; Batty et al., 2010), especially reduced species richness and abundance of mayfly larvae (order Ephemoptera), associated with decline in numbers or often complete absence of specific sensitive species like ephemerellid and heptageniid mayflies (Clements, 1991, 2000; Gower et al., 1994; Cain et al., 2004; Luoma and Rainbow, 2008). Fish are typically of low abundance with fish mortalities, particularly of more sensitive salmonid species (e.g. rainbow trout *Oncorhynchus mykiss* in the Clark Fork River, US: Marr et al., 1995), reducing the

number of fish species, and clogging by metal-rich sediment may cause loss of spawning gravels and nursery streams for fish reproduction (Malouf, 1974; Environment Agency, 2008b; Luoma and Rainbow, 2008).

Historically, amongst the earliest observations of the ecotoxicological effects of mining activities on British water catchments was the work of Carpenter in the 1920s in the then active mining regions of West Wales (Carpenter, 1924; Kelly, 1988; Environment Agency, 2009). Carpenter (1924) thus noted that the flora in lead and zinc contaminated streams immediately downstream of mines was very reduced, the flora being typically restricted to growths of species of the red algal genera *Batrochospermum* and *Lemanea*, together with some mosses and liverworts (Kelly, 1988). Floral species number increased downstream and over time in the upper reaches after closure of the mines (Kelly, 1988).

In the late 1970s and 1980s, Whitton, Say and co-workers researched the ecotoxicological effects of mining contamination in streams and rivers of the Northern Pennine area where zinc, cadmium and lead are the major contaminants; this area is underlain by Carboniferous limestone with high buffering capacity counteracting confounding ecotoxicological effects of low pH in the local streams (Say and Whitton, 1981a, b; Environment Agency, 2009). Say and Whitton (1981a) found only 25 photosynthetic species in a heavily zinc-contaminated stream while a nearby uncontaminated stream housed 61 species. It is the loss of species (for example *Cladophora* species – Whitton, 1970) in these examples that then allows mass growths of more metal-resistant species to flourish in the absence of competition (Environment Agency, 2009). Armitage (1979) found reduced numbers of taxa and individuals in the benthic fauna of sites with very high water zinc levels (>2mg l^{-1}) in the River Nent system in the Northern Pennine orefield, but faunal abundance and diversity were also lower at points with relatively low dissolved zinc concentrations indicating that other factors may influence the fauna. Low benthic fauna numbers were associated with a massive growth of the alga *Stigeoclonium tenue*, a species resistant to both high zinc availabilities (Harding and Whitton, 1976) and organic pollution, and it was difficult to separate the effects of high zinc on benthic faunal abundance from those resulting from algal growth and the physical characteristics of the substratum (Armitage, 1979).

In the 1990s Gower and co-workers investigated in detail the effect of metal contamination derived from mining on insect assemblages in the many metal-rich streams of Devon and Cornwall in SW England (Gower and Darlington, 1990; Gower et al., 1994). Other case study examples will be given below in descriptions of the different monitoring techniques used in the environmental impact assessment of metals derived from mining on the communities of affected streams.

1.3.4 Principles of community ecotoxicology

More than 25 years ago, John Cairns Jr. highlighted the lack of the integration of ecological theory to provide a sound ecological basis in the emerging field of ecotoxicology (Cairns, 1986; Clements and Rohr, 2009). The situation is better today, though still not yet ideal (Newman and Clements, 2008; Schmitt-Jansen et al., 2008; Luoma and Rainbow, 2010; Newman, 2010). Of particular relevance here, has been the emergence of a new discipline, that of *community ecotoxicology* defined as the study of the effects of contaminants on patterns of species abundance, diversity, community composition and species interactions (Clements and Newman, 2002; Clements and Rohr, 2009). Because communities lie between populations and ecosystems in the hierarchy of levels of biological organization, they offer important insights into the mechanisms of contaminant action at lower levels while being closely connected to relevant endpoints, such as the provision of ecosystem services, at higher levels. Furthermore, species diversity is positively associated with ecosystem stability and recovery, and thus a community-level perspective on ecotoxicology is more important than ever (Clements and Rohr, 2009). Community ecotoxicology will have come of

age when it shifts from a purely descriptive to a predictive science (Clements and Rohr, 2009).

a. Community level biological monitoring

In their book *Community Ecotoxicology*, Clements and Newman (2002) present an excellent introduction to different methods of assessing biological changes at community level, such as those caused by the ecotoxicological action of mine-derived metals in stream habitats. Community-level biological monitoring is based on the assumption that the composition and organization of communities reflect local environmental conditions and respond to any significant anthropogenic alterations to these conditions (Clements and Newman, 2002). A second assumption is that species differ in their sensitivity to anthropogenic stressors which thereby cause structural changes in community composition (Clements and Newman, 2002).

Given the assumption that species differ in their sensitivity to the presence of toxic metals at raised bioavailabilities, then an ecotoxicologically significant dose of a metal in a habitat will change the numbers of species present and the abundances of those remaining. Measures of the numbers of species present and their abundance have typically been the first steps in any quantitative analysis of the community structure of, for example, benthic invertebrates in streams (e.g. Malmqvist and Hoffsten, 1999). *Species richness* is defined as the number of species present in a prescribed sampling unit (Clements and Newman, 2002). Although an apparently simple measure, species richness is highly dependent on sampling effort (for example the area sampled) and increases asymptotically with sample size and number of individuals collected, eventually levelling off as we approach the point when all species have been recorded. Hence, a standardized sampling protocol (e.g. RIVPACS 3-minute kick sample) is necessary when comparing across sites.

A particular problem, however, of measures of species richness is that they do not take into account differences in abundance among the species present. Two compared habitats may have the same number of species but differ greatly in the abundances of the species present. Even in the absence of anthropogenic contamination, species differ in their abundance in habitats. Some species are rare while others are very abundant, and species abundance models have been developed to quantify species abundance distributions. Such species abundance models are useful to summarise data from community surveys. Models are fitted to observed species abundance data and parameters of the model have value as summary statistics for the data set, incorporating both species number and abundance data. In practice four abundance models are commonly used – logarithmic series, geometric series, discrete lognormal and broken stick (Clements and Newman, 2002) – and the model that fits the observed data best is chosen to be used in further analysis. The discrete lognormal model fits most communities and is often advocated as a universally acceptable species abundance model (May, 1976; Clements and Newman, 2002).

Measures of *species diversity* have been developed to provide a single value that incorporates information on both species richness (the number of species) and species abundances. Species diversity indices are often used in biological monitoring studies to compare communities in different locations (e.g. Amisah and Cowx, 2000; Hirst, Jütner and Ormerod 2002; Gray and Delaney, 2008), although they have been criticised on theoretical, statistical and conceptual arguments (Clements and Newman, 2002). Simpson's Index, in original or modified form, is based on dominance, being very sensitive to dominant species and relatively insensitive to rare species. Clements and Newman (2002) propose that two other diversity indices based on information theory, the Shannon-Wiener and Brillouin Indices, which are more sensitive to rare species, are more relevant to ecotoxicology. The Shannon-Wiener Index is in effect a measure of the uncertainty of predicting the species of a randomly chosen individual from the community. The Shannon-Wiener Index estimates

diversity for the community from which the sample was taken, whereas the Brillouin Index estimates diversity for the sample itself (Clements and Newman, 2002).

Another measure of community structure is *species evenness*, a measure of how equally the individuals in a community are distributed among the species present. For example Pielou's evenness index is based on a comparison between the measured value of the Shannon-Wiener Index and its estimated maximum value. It is constrained to a value between 0 and 1, where 1 represents maximum evenness.

Single number indices are described as univariate, and although simple, they do have the serious deficiency of loss of information that inevitably occurs when details of community composition are reduced to a single number. While classic measures of benthic invertebrate community structure in freshwater habitats involved univariate indices, multivariate statistical techniques are used more frequently today (Sparks, 2000; Henderson and Seaby, 2008). Many multivariate techniques aim to replace the original large set of variables by a much smaller set of derived variables (often referred to as axes) which still retain most of the relevant information – a process termed ordination (Scott and Clarke, 2000). Ordination tries to present results by plotting graphs of the new variables against each other, allowing us to visualise relationships between sites, samples, species, variables, etc. Principal Components Analysis (PCA) was the first ordination technique to be developed and is still commonly used (e.g. Beltman et al., 1999). PCA chooses variables (principal components) that embody as much as possible of the variance in the data, calculating components in sequence until all of the original variance is accounted for. PCA was developed to analyse continuous quantitative data, but Correspondence Analysis (CA) can be used with non-continuous (categorical) data where the numbers 1, 2, 3 etc. are assigned to categorical data, for example shapes or colours. CA can in fact be used for any dataset (Scott and Clarke, 2000) since continuous variables can be assigned to categories, as for example in defining bioaccumulated metal concentrations as high, medium or low with defined cut-off points. Variants of CA include Canonical Correspondence Analysis (CCA) (e.g. Gower at al., 1994) and De-trended Correspondence Analysis (DCA) (e.g. Gower et al., 1994; Hirst et al., 2002). Multidimensional Scaling (MDS) is another ordination technique, becoming increasingly popular in aquatic ecological studies (Clarke and Ainsworth, 1993). MDS produces a graphical representation of the similarity between samples in a small number of dimensions, this similarity being measured, for example, in terms of species abundance or presence/absence data (Henderson and Seaby, 2008). The choice of similarity measure varies, and it is common to use both similarity measures requiring presence/absence data such as the Sorensen and Jaccard Similarity Indices and measures using quantitative data such as Bray-Curtis (Henderson and Seaby, 2008). MDS analyses the matrix of pair-wise similarities between all the samples in the data set, achieving the typical aim of a biological monitoring survey in distinguishing between sites or sampling occasions. There are two forms of MDS, metric and non-metric, the latter being particularly popular for it can be used with non-parametric data such as ranks (Scott and Clarke, 2000).

b Relationships between variables

Moving beyond comparisons of communities, it is often an aim to relate species compositions to environmental variables, for example water or sediment metal concentrations. Ordination techniques are available in which the axes are constrained to represent the relationships between two sets of variables (typically community composition and environmental variables), sometimes referred to as direct gradient analysis (Scott and Clarke, 2000). The two sets of variables are represented on the same ordination diagram and assessment of relationships is made directly from this. Gower et al. (1994) used CCA to show that variation in macroinvertebrate communities in metal-contaminated streams in south-west England was best explained by four stream chemistry variables (Cu, Al, alkalinity, pH). ; Byrne et al. (2013b) used the same approach to detect metal effects in

Wales. Another way of examining relationships between response and explanatory variables is multivariate multiple regression, not a form of ordination. In this case several response variables are involved and the result of the analysis is a set of regression equations, one for each response variable, but taking into account interrelationships between the response variables as well as their relationship to the explanatory variables (Scott and Clarke, 2000). Redundancy Analysis (RDA) falls between ordination techniques and multivariate multiple regression, and can be used to determine the relative importance of anthropogenic factors like metals and other environmental factors in explaining the variability in community composition data, for example macroinvertebrate and diatom community compositions in a study of metal pollution in a lowland river in Flanders (De Jonge et al., 2008).

Linear regression models are often not appropriate for linking environmental variables with changes in communities. Theoretical and empirical studies do indicate that some communities show abrupt, non-linear changes in structure or function in response to perturbations (May, 1977; Clements and Rohr, 2009; Luoma et al., 2010; Rainbow et al., 2012; Schmidt et al., 2013). These ecological discontinuities or thresholds are defined as significant changes in an ecological state variable as a result of a continuous change in an independent environmental variable (Clements and Rohr, 2009).The threshold is the point at which any rapid change initially occurs. Typically, in any ecological study assessing the effects of contaminant stressors on resident communities, not all the factors that might affect the communities are measured, and statistical distributions of ecological data have unequal variation as a result of complex interactions between these factors (Cade and Noon, 2003; Schmidt et al., 2012). Such unequal variation can be examined by quantile regression analysis which estimates multiple rates of change (slopes) from the minimum to maximum response, and provides a more complete picture of the relationships between variables missed by other regression methods (Cade and Noon, 2003). Schmidt et al. (2012) used quantile regression to measure risks to aquatic life exposed to metals in a study of the population densities of two mayflies and a caddisfly, metals and other environmental variables in 125 streams in Colorado, USA. In accordance with the concept of ecological thresholds, the most obvious effects on mayfly populations were at upper quantiles and not mean density (Schmidt et al., 2012).

1.3.5 Biotic indices

a Macroinvertebrate biotic indices

Another approach taken to overcome the deficiencies of univariate measures of community diversity has been the development of biotic indices (Metcalfe, 1989; Clements and Newman, 2002). The community based indices above give all species equal weight irrespective of their sensitivity to anthropogenic contamination, and may not show up responses if, for example, sensitive species are simply replaced by contaminant-insensitive species. Different *biotic indices* have therefore been developed to assess the state of the community based on the relative abundance of sensitive and resistant species, particularly in the case of *benthic macroinvertebrates* in streams and rivers. It follows that different species show different relative sensitivities according to the contaminant concerned, and so it should not be surprising if a biotic index relevant to the assessment of organic pollution is not applicable to an assessment of toxic metal pollution, and vice versa. Nevertheless, relationships between the extent of metal pollution and indices related to organic pollution (Gehardt et al., 2004; De Jong et al., 2008, 2013; Blanco and Bécares, 2010), and measures of invertebrate diversity and richness (Hirst et al., 2002, De Jong et al., 2008, 2013; Blanco and Bécares, 2010) have been recorded.

[Many descriptions of the development and use of biotic indices use the terms contaminant or pollution-tolerant when referring to the attributes of particular taxa at whatever taxonomic level, typically family, genus or species. In this review, the term contaminant-insensitive is

preferred when referring to the particular characteristics of a taxon irrespective of particular local habitat, and contaminant-tolerant is used (see later) to refer to the characteristics of a particular exposed population, whether or not that tolerance is inheritable after selection over several generations or has been derived by physiological acclimation and is restricted to the one exposed generation (Amiard-Triquet et al., 2011).]

The simplest expression of such an approach lies in the concept of indicator species. An *indicator species* is a species the presence or absence of which is indicative of a particular habitat, community or set of environmental conditions. In an ideal world there would exist a species whose presence or high abundance would indicate a high local bioavailability of toxic metal(s) in a particular habitat, in our case a freshwater stream. Indeed the indicator species concept was popular with freshwater ecologists who recognised the large number of pollution-insensitive chironomid larvae (midges – Diptera, Chironomidae) and oligochaete worms such as tubificids replacing more sensitive mayflies (Ephemeroptera) and stoneflies Plecoptera) at sites with high organic enrichment (Clements and Newman, 2002). There are, however, severe limitations with the indicator species approach. Firstly there is the inherent tautology of defining a pollution-sensitive species as a species absent from polluted habitats. Secondly there is the greater problem of the need to distinguish the relative significance of an anthropogenic stressor from the effects of the many other biotic and abiotic factors that influence the presence or absence of a species (Clements and Newman, 2002): the alga *Stigeoclonium tenue* present at high abundance in the zinc-rich streams of the River Nent system is relatively insensitive to other forms of pollution as well as high zinc levels (Armitage, 1979). In freshwater systems, so-called pollution-sensitive species are also sensitive to other disturbances, natural or anthropogenic, and the absence of such a species provides only weak support for the hypothesis that its absence is a result of the local presence of a particular toxic contaminant (Clements and Newman, 2002).

The biotic indices relevant to the analysis of the community structure of benthic freshwater flora and faunas are often based on the relative abundance of species decreed by expert opinion to be sensitive or to a specific type of pollutant or not, but make use of much contributing data on the relative abundances of these species. It must be remembered, however, that relative sensitivities of species do depend on the contaminants of concern and many of the biotic indices developed for use in freshwater systems are particularly appropriate for the assessment of the comparative effects of organic enrichment. It is well known that mayflies (Ephemoptera), stoneflies (Plecoptera) and caddisflies (Trichoptera) are relatively sensitive to organic enrichment, while chironomids (Diptera) are generally resistant to organic pollution. The *EPT score* (Lenat, 1988) makes direct use of these relative sensitivities to create a biotic index (e.g. Malmqvist and Hoffsten, 1999; Gray and Delaney, 2008).

In the UK in the 1960s the then Trent River Authority developed the *Trent Biotic Index (TBI)* to assess (organic) pollution in freshwaters (Metcalfe, 1989), based again on the presence/absence of mayflies, stoneflies and caddisflies, but also of amphipod and isopod crustaceans, tubificid oligochaetes and chironomid larvae red with haemoglobin (Woodiwiss, 1964). The total number of groups present is cross-referenced against a hierarchy of indicator taxa from sensitive to increasingly resistant until not even oligochaetes or chironomids are present. The index is simple but no account is taken of abundances and a single specimen of a sensitive species drifted down from a less organically rich region upstream will bias the calculation of the index at a downstream site (Jeffries and Mills, 1990). The TBI paved the way for a number of indices across the world adapted to the local fauna (Metcalfe, 1989), such as the *Indice Biotique* and later the *Indice Biologique de Qualité Génerale (IBG)* in France (Tuffery and Verneaux, 1968; Verneaux et al., 1982), the *Belgian Biotic Index (BBI)* (De Pauw and Vanhooren, 1983), and *Hilsenhoff's Biotic Index* in the USA (Hilsenhoff, 1987). Hilsenhoff's Biotic Index also takes advantage of differences in relative sensitivities of taxa to come up with a value based on the relative abundance of sensitive

and tolerant species, originally using data from more than 2000 macroinvertebrate samples from organically polluted and unpolluted Wisconsin streams. In the UK, in a development from the TBI, the *Chandler score* (Chandler, 1970) incorporates abundance data (Metcalfe, 1989), as used by Armitage (1979). Taxa are again ranked in a hierarchy from sensitive to resistant, and scores are given for presence but varied across five abundance categories to produce a total score. Superseding the Chandler score and still widely used in the UK today is the *Biological Monitoring Working Party (BMWP) score* (Biological Monitoring Working Party, 1978), produced by a working party convened in 1976 by the UK Department of the Environment (Metcalfe, 1989; Jones et al., 2010). Operation of the BMWP score is simpler than that of the Chandler score for it does not try to include abundance data. Taxa are still ranked according to their sensitivity to organic pollution with corresponding scores from 10 (sensitive) to 1 (resistant) which are summated to give the total score. A positive point of the BMWP score is the improved precision of taxon resistance and the taxonomic level to which identification is taken (Jeffries and Mills, 1990). Rather than using the BMWP score for a sample, the Average Score Per Taxon (ASPT) is used, derived by dividing the BMWP score by the number of taxa used in its generation: ASPT is less sensitive to variation in sampling effort than is BMWP, and therefore provides a more consistent measure of the level of pollution at a site. A high ASPT reflects the presence of many sensitive species (Jeffries and Mills, 1990). In addition the number of scoring families (NTAXA) in calculating the BMWP score may also be quoted (Jones et al., 2010). Recently the BMWP scoring system has been revised, and a system to incorporate abundance weighted scores developed, with the scores based on regression analysis relating the occurrence of taxa to estimates of pollution: the new scoring system named WHPT after its originators, Walley, Hawkes, Paisley and Trigg (Paisley, Trigg and Walley, 2014).

The BMWP score was developed as an index to assess the ecological effects of organic pollution (Jones et al., 2010), and it cannot therefore be expected from first principles to be suitable as a biotic index for mining effluent pollution. Nevertheless, the use of BMWP has been evaluated in this context, specifically with respect to coal mine water discharges (Davies et al., 1997; Jarvis and Younger, 1997, 2000; Environment Agency, 2009) and acid mine drainage (Gray and Delaney, 2008). The ecological impact of coal mine water discharges was assessed by measuring decreases in BMWP score downstream compared with upstream of a coal mine discharge, the extent of any ecological impact being assessed by identifying the downstream point at which the BMWP score returned to its upstream value (Environment Agency, 2009). In fact the BMWP score worked well for coal mine water discharges for which the deposition of iron oxides/hydroxides is the major cause of depletion of fauna (Environment Agency, 2009), and Jarvis and Younger (1997) had already shown a strong statistical inverse correlation between BMWP score and iron concentration in affected streams. It may be the physical blanketing by the ochre and associated reduced light penetration that are the main causes of decreases in faunal abundance and diversity (Environment Agency, 2009), as opposed to any toxic metal effect that might be more typical of mining-affected streams with metal-rich sediments. In the case of discharges from metal mines, any relationships between dissolved concentrations of metals such as zinc, lead and cadmium, and BMWP scores were far more ambiguous than for coal mine drainage, with no clear strong inverse relationships present (Environment Agency, 2009; Jarvis and Younger, 2000). A specific study of streams in the mining-affected regions of Gwynedd and Ceredigion in Wales drew similar conclusions; there was a correlation between BMWP score (but not ASPT) and total zinc concentration, albeit with considerable scatter in the data, but no clear relationship between any other trace metal (arsenic, cadmium, copper, iron, manganese, nickel, lead, as total or dissolved fraction) and BMWP score, ASPT or number of taxa present (Environment Agency, 2009). Furthermore in this study there was a potentially strong confounding effect present, in the form of a strong inverse relationship between pH and BMWP score. The Environment Agency report (Environment Agency, 2009) therefore questions the appropriateness of Environmental Quality Standards (EQS) for streams that have long been impacted by metal mine discharges. Furthermore, the potential

inappropriateness of the BMWP score for use as a biotic index in mining-affected streams is probably also a factor producing the reported lack of relationships investigated.
Gray and Delaney (2008) did find the BMWP to be an excellent index of AMD in the River Avoca in Ireland draining a region of abandoned copper sulphide mines, showing significant reduction in both abundance and taxon richness of benthic macroinvertebrates in response to AMD. The effects of AMD are, however, driven by a number of factors, particularly water acidity, in addition to salinization, metal toxicity and metal precipitation, confounding the direct ecotoxicological effects of metal-rich sediments. On the other hand the ASPT derived from the BMWP was not correlated with any measured parameter such as pH, sulphate, zinc or iron concentrations, nor, unlike the BMWP score, with any of the diversity indices measured (Gray and Delaney, 2008). Gray and Delaney (2008) concluded that specific biotic indices do need to be developed that measure the expected community structure of macrobenthic invertebrates arising from AMD impact more precisely than the existing metrics.

The biotic indices discussed above are not particularly useful as stand-alone measures as there is natural variation in the occurrence of species and, hence, index scores vary naturally dependent upon the natural characteristics of the river being investigated. To assess the extent of pollution at a site, it is necessary to determine if the community structure of benthic macroinvertebrates (or other biota) deviates from the community that would be expected in the absence of pollution. The *River InVertebrate Prediction and Classification System (RIVPACS)* (Wright et al., 1984) represented a major step forward because it adopted a 'reference condition' approach (Jones et al., 2010). The fauna at a site under investigation is compared with fauna at similar 'reference sites' that are not subject to any apparent environmental stress, site quality being measured as a ratio (the observed/expected score) where the expected score has been predicted by RIVPACS based on the fauna at similar sites with matching physical, chemical and geographical characteristics (Jones et al., 2010). Thus an extensive dataset was compiled of macroinvertebrate assemblages (identified to species level) from (initially) 268 representative sites across the UK not subject to pollution or other environmental stress. The 642 species or species groups in the reference dataset were used to divide the sites statistically into initially 16 end groups based on the similarity of their fauna. Multiple discriminant analysis was then used to identify the best set of physical, chemical and geographical predictor variables to separate these different biological end groups, eventually reducing the physico-chemical variables measured to 11 pollution-insensitive predictor variables that are then used to match a test site to the most appropriate reference biological end group (Jones et al., 2010). RIVPACS became the main tool used by regulatory authorities in the UK for the biological monitoring of rivers. In practice, an expected score is derived for the average BMWP score per taxon (ASPT) and for the number of BMWP scoring families (NTAXA), and compared with the observed scores derived from a family level identification of samples from the test site (Jones et al., 2010). Although RIVPACS was developed for the assessment of organic pollution (based on BMWP, ASPT and NTAXA), and not specifically for the assessment of the ecotoxicological effects of trace metals derived from mining, the RIVPACS models produce predictions of the invertebrate community expected at a site in terms of the species present and their (log) abundance (Jones et al. 2010). Nevertheless, De Jonge et al. (2013) demonstrated a negative relationship between dissolved metal concentrations and the observed/expected of the indices NTAXA and BMWP, and invertebrate metal body burdens in rivers in northern England.

In 2008, the Water Framework Directive United-Kingdom Advisory Group (WFD-UKTAG) published a method statement for monitoring, assessing and classifying the condition of benthic invertebrates in rivers in England, Northern Ireland, Scotland and Wales in accordance with the Water Framework Directive. This method is based on RIVPACS methodology housed in a software tool known as the *River Invertebrate Classification Tool (RICT)* (WFD-UKTAG, 2008b). At that time RICT outputs focussed on the impact of organic

enrichment on the condition of the WFD quality element 'benthic invertebrates' (as listed in Table 1.2.1 of Annex V to the Water Framework Directive), using the indices Number of Taxa (NTAXA) and Average Score per Taxon (ASPT), and provided a list of benthic invertebrate taxa to be recorded (WFD-UKTAG, 2008b). These measured indices were compared against reference values for each index derived for reference sites (identified by Environmental Variables) producing an Ecological Equality Ratio (EQR) for each of the two indices (WFD-UKTAG, 2008b). More recently, the Walley Hawkes Paisley Trigg Method (WHPT) for assessing river invertebrate communities represents a technical development of BMWP, incorporating a revision of the taxonomy of the scoring taxa, inclusion of more families, and the addition of an abundance weighting scheme (Walley and Hawkes, 1996, 1997; SNIFFER, 2011a). WHPT again addresses the impact of organic enrichment on the composition of the benthic macroinvertebrate communities of affected rivers. Nevertheless, in its current form RICT is set up to calculate a variety of indices assessing a range of stressors (Davy-Bowker et al., 2008; Clarke et al., 2011), with the reference dataset screened to ensure that the sites were not suffering from stress at the time of sampling (Davy-Bowker et al., 2007) and, therefore, serve as an adequate reference for novel stressors not yet included in the outputs.

Another development, the WFD Acid Water Invertebrates Classification Tool (WFD-AWIC), addresses the effects of acidification on river benthic invertebrate communities, using a reduced list of taxa known to respond to acidification (Davy-Bowker et al., 2005; SNIFFER, 2011a). Initially the tool used family level taxa ($AWIC_{fam}$), but subsequent developments of AWIC have used species level identifications ($AWIC_{sp}$) and abundance-weighted species data (WFD $AWIC_{sp}$) (SNIFFER, 2011a). Davy-Bowker et al. (2005) concluded that, at family level, benthic macroinvertebrate communities are not characterised by a specific obligate community inhabiting acid waters. Acid sites do support invertebrate families that are acid-tolerant but these same families are also found in circum-neutral waters. $AWIC_{fam}$ works by distinguishing sites on the absence of many acid-sensitive families, and the presence of any one family can shift the index considerably (SNIFFER, 2011a). Ormerod et al. (2006) investigated the performance of $AWIC_{fam}$ in 132 Welsh and Scottish streams and the index values did correlate with expected physical variables including pH, with the potential, therefore, to replace frequent measurements of pH. Recommendations made by Ormerod et al. (2006) were applied in the subsequent $AWIC_{sp}$ and WFD $AWIC_{sp}$ indices (SNIFFER, 2011a; Murphy et al. 2013). $AWIC_{sp}$ outperforms $AWIC_{fam}$ and is a powerful species-level index that effectively discriminates sites with low pH (SNIFFER, 2011a; Murphy et al. 2013) and has been used to great effect in determining the extent of recovery of acidified sites (Murphy et al. 2014). The incorporation of abundance weighting has made WFD $AWIC_{sp}$ more compliant with the WFD requirement to assess the abundance of biological quality elements, and WFD $AWIC_{sp}$ does offer a marginally improved performance over $AWIC_{sp}$ (SNIFFER, 2011a; Murphy et al. 2013).

In Australia a diagnostic index for acid mine drainage, SIGNAL-MET, was developed where taxa were scored using correlation with distance downstream of mine discharges (Chessman and McEvoy, 1998). This index (which is indicative of the various stressors associated with mining activity) has been tested using an Australian predictive model, AUSRIVAS, which is based on RIVPACS (Sloane and Norris, 2003). 70% of the variation in observed/expected was explained by variables associated with mine pollution (copper, cadmium, lead and zinc in the sediment, cadmium and zinc in the water, and pH). Sloane and Norris concluded that the predictive modelling (RIVPACS) approach is appropriate for assessing the degree of impairment from previous mining activity.

Although previously only used to assess organic pollution, RIVPACS models can produce expected values for different biotic indices, enabling assessment of other stressors such as acidity (as addressed above by AWIC). LIFE (Lotic-invertebrate Index for Flow Evaluation), PSI (Proportion of Sediment sensitive Invertebrates), CoFSI (Combined Fine Sediment

Index) and $SPEAR_{pesticides}$ (SPEcies At Risk of pesticides) are such indices, all being based on a classification of taxon sensitivity to determine the proportion of individuals in a sample that are sensitive to a particular stressor. LIFE seeks to link changes in river benthic invertebrate communities to prevailing flow regimes (Extence et al., 1999). Both family and species-level LIFE indices are abundance-weighted and their performances are impressive in relating various flow variables to changes in the indices in a range of different stream types and hydrologies (Monk et al., 2006; SNIFFER, 2011a). Family and species level PSI indices are designed to relate benthic macroinvertebrate community changes to fine sedimentation stress (Extence et al., 2013). Allocation of taxa to a Sediment Sensitivity Ranking (SSR) includes consideration of sediment-sensitive (negative) traits (e.g. blocking of collecting nets of hydropsychid caddisflies or loss of algae as a food resource to scrapers), as well as sediment-insensitive (positive) traits (e.g. provision of habitat for burrowing oligochaetes). A further fine sediment index (CoFSI) which returns an average score (akin to ASPT and less prone to sampling variation), was developed using objective statistical techniques where species were scored based on empirical analysis of their distributions in response to excess deposited fine sediment, and represents the combined response to organic and inorganic deposited fine sediment (Murphy et al. 2015). The family and species-level $SPEAR_{pesticides}$ indices are designed to reflect pesticide contamination in running waters, and have been applied in the field in Europe and the UK with success (Liess and Von der Ohe, 2005; Schäfer et al., 2007; Beketov et al., 2008, 2009; Liess et al., 2008a, b; SNIFFER, 2011a).

In spite of these developments of RICT, as concluded by SNIFFER (2011a), there still exists a need to develop a biotic index for metal pollution.

b Sediment Quality Biotic Indices

Whilst the Australian SIGNAL-MET index is associated with the various impacts of mining, not just metal-rich sediments, in both France and Belgium there have been initiatives to develop biotic indices of sediment quality in rivers, the *Oligochaete Index of Sediment Bioindication (IOBS)* in France (Rosso et al., 1994) and the *Biotic Sediment Index (BSI)* in Belgium (De Pauw and Heylen, 2001; De Pauw et al., 2002).

In France, the IOBS aims to assess the general quality of stable sediments in watercourses, by calculating the percentage of species in the oligochaete worm family Tubificidae in the total number of oligochaete species found. The larger the percentage of tubificids, the lower is the index score - interpreted as poor quality sediment, particularly in terms of organic enrichment. The proportion of tubificids that have setae (hairs) is also assessed: the lack of setae is taken to be an indication of pollution of the sediments by toxic metals and organic contaminants (PCBs) (Rosso et al., 1994; Prygiel et al., 2000). Thus, Prygiel et al. (2000) used the IOBS to assess the ecotoxicological status of sediments of rivers and canals in the Artois-Picardie water basin. They concluded that there was an inverse relationship between the IOBS score and sediment trace metal concentrations, and a correlation between these concentrations and the percentage of tubificids without setae (Prygiel et al., 2000). The IOBS, thus shows promise as a biotic index of the ecotoxicological effects of metals in sediment, although this study (Prygiel et al., 2000) was confounded by the additional contamination of the sediments by polyaromatic hydrocarbons (PAH) and PCBs. Furthermore, it should be noted that this index requires a far higher degree of taxonomic resolution than is typically used in the UK, where Oligochaeta are rarely taken above Class.

In Belgium, the BSI has been developed from the Belgian Biotic Index (BBI). The BSI is based on the taxonomic diversity of the benthic macroinvertebrate community in a given sediment sample and the presence or absence of indicator taxa reflecting different degrees of pollution tolerance (De Pauw and Heylen, 2001; De Pauw et al., 2002).

More recently Archaimbault et al. (2010) investigated the ecological quality of stream sediments from sites on 150 mountain streams in France again using benthic macroinvertebrate communities. Sites were pre-assigned to one of four toxic quality classes ranging from high to poor on the basis of concentrations of toxic substances (trace metals, PAH and PCB) in the sediment, following the French environmental water quality assessment system. A non-parametric multiple comparison statistical procedure was used to compare relative abundances of different biological traits of the macroinvertebrates (e.g. habitat preference, contaminant resistance, biogeographic distribution, etc.) between groups of sites assigned to the different four toxic quality classes above, in order to identify the combinations of traits that best separated sites between adjacent toxic quality classes. Archaimbault et al. (2010) were ultimately able to allocate sites to toxic quality classes from the biological attributes of their invertebrate communities with confidence, and their tool has considerable potential, after development, as an *in situ* functional tool of stream sediment contamination assessment at community level.

c Other biological quality elements of the Water Framework Directive

Benthic macroinvertebrates have been the target community in the biotic indices discussed above, but WFD legislation stipulates that several different biological quality elements need to be assessed in addition to invertebrates. Phytoplankton, phytobenthos (macrophytes and benthic algae) and fish are to be considered depending on the type of water body (European Parliament, 2000). New tools have been developed for UK rivers in response to these needs of the WFD (Environment Agency, 2009) – DARLEQ (Diatoms for Assessing River and Lake Ecological Quality) which uses diatoms as proxies for benthic algae (Kelly et al., 2008), LEAFPACS (WFD-UKTAG, 2009) which uses macrophytes, and FCS2 (Fisheries Classification Scheme 2; SNIFFER 2011b) for fish. The original DARLEQ is calibrated against a nutrient-organic gradient, as for so many macroinvertebrate biotic indices above, and so is not designed specifically to detect toxic effects (Environmental Agency, 2009). LEAFPACS is a multimetric tool designed to assess nutrient/organic and general degradation effects, again not for assessment of contaminant ecotoxicological effects (Environment Agency, 2009). FCS2 is a non-parametric (smooth) geostatistical model which compares the catch of the 23 most prevalent fish species to a predicted catch, but is not specific to any stressor (SNIFFER, 2011b).

The Environment Agency Report on ecological indicators for abandoned mines (Environment Agency, 2009) considered whether DARLEQ or LEAFPACS could be developed for the assessment of sensitivity to metal effects. DARLEQ is being developed to produce an acidification metric (Diatom Acidification Metric DAM), and there is potential for metrics testing for reductions in diatom diversity observed at high metal concentrations to be added (Environment Agency, 2009). Hirst et al. (2002) investigated the responses of diatom communities to dissolved metal concentrations in Welsh and Cornish streams in metal-mining areas, but showed that changes in pH and conductivity best explained variations in diatom assemblage compositions. Species diversity, species richness and evenness did not vary with metal concentrations (Hirst et al., 2002). On the other hand, the single strongest predictor of the structure of diatom assemblages was the Cumulative Criterion Unit (CCU) score, a measure of total stream metal concentration and toxicity (see later). Diatom species apparently tolerant of high metal concentrations included *Psammothidium helveticum*, *Eunotia subarcuatoides*, *Pinnularia subcapitata* and *Sellaphora seminulum*, with interspecific differences in the pH ranges in which they were abundant (Hirst et al., 2002). Sensitive species included *Fragilaria capucina* var. *rumpens*, *Achnanthes oblongella* and *Tabellaria flocculosa* (Hirst et al., 2002). De Jonge et al. (2008) included diatom communities in their biological assessment of a gradient of metal pollution in the River Dommel in Belgium. Significant variables explaining diatom community structure were conductivity (16.5%), chloride (11.4%), ammonium (10.6%) and zinc (5.9%), and the diatom community structure better reflected the metal gradient than the macroinvertebrate community structure (De

Jonge et al., 2008). Three different groups of diatoms could be separated in relation to metal concentrations: *Tabellaria flocculosa* and *Flagellaria capucina* var. *rumpens* were (again) associated with low metal concentrations, *Gomphonema parvulum* and *Nitzschia palea* with elevated metal concentrations, and *Sellaphora seminulum* (again) and *Eolimna minima* with high zinc concentrations (De Jonge et al., 2008). Blanco and Bécares (2010) found good correlations between existing diatom based indices (mostly developed for assessing nutrient pollution) and dissolved metal concentrations, but again little impact on diversity. It does therefore appear that a biotic index based on diatom community compositions may well be a valid assessment of the effects of high metal bioavailabilities in streams affected by mining, albeit that the evidence so far concerns dissolved metal concentrations.

Although mosses and liverworts may be sensitive to metal pollution, LEAFPACS appears to show less potential than DARLEQ for development as a biotic index for metal pollution, not least because of the relative lack of macrophytes in upland streams typically affected by mining and the difficulty of accurate specific identification of filamentous green algae (Environment Agency, 2009).

Fish are listed as a biological quality element of the Water Framework Directive. Predatory fish are typically of low abundance in mine-impacted streams and fish that are present are often small or have shortened life spans (Luoma and Rainbow, 2008).

In the metal-contaminated Clark Fork River system and other mine-impacted North American streams, it is not unusual to find only the most metal-tolerant species of trout, the brown trout *Salmo trutta*, although the habitat might otherwise be expected to contain additionally rainbow trout *Oncorhynchus mykiss*, cutthroat trout *Oncorhynchus clarki lewisi*, bull trout *Salvelinus confluentus*, and brook trout *Salvelinus fontinalis* (Luoma and Rainbow, 2008). Even brown trout are absent from extremely metal-contaminated streams. It appears that the ingestion of metal-contaminated prey is the significant source of stress to the fish, as exemplified by brown trout fed on metal-rich invertebrates from metal-contaminated regions of the Clark Fork River showing elevated biochemical dysfunctions, histological abnormalities, reduced growth and survival (Farag et al., 1994; Woodward et al., 1994, 1995; Luoma and Rainbow, 2008).

In the UK, a study of pollutants from the coal industry affecting a small river in the South Wales coalfield showed that introduced brown trout *Salmo trutta* occurred at very low densities downstream of acid and ferruginous drainage, in the latter case apparently as a result of impoverished food supply (Scullion and Edwards, 1980).

Maitland (2004) produced a commissioned report for Scottish Natural Heritage on the ecological and conservation status of freshwater fish communities in the UK, exploring the potentially valuable interrelationships between SERCON (System for Evaluating Rivers for Conservation) and the Water Framework Directive. SERCON was designed to assess the naturalness of fish communities, being concerned with the intrusion of alien fish species into freshwater systems (e.g. Boon et al., 2002), and the experience of SERCON has the potential to be incorporated into an assessment of ecological status to meet WFD objectives (Maitland, 2004). Nevertheless no biotic index based on freshwater fish communities has yet to be developed that might be applicable for an ecological assessment of the ecotoxicological effect of metal-rich sediments in streams, not least as a result of the very simple nature of fish communities that might be expected to occur in appropriate reference sites.

While *meiofauna* are not specifically identified as a biological quality element of the Water Framework Directive, Burton et al. (2001) did investigate the relationship between the composition of freshwater meiofaunal communities and trace metal contamination, again in the streams of Cornwall, using univariate and multivariate statistical techniques including

non-metric MDS. Dissolved copper concentration (singly or in combination with aluminium, zinc or dissolved organic carbon) was the most important correlate with meiofaunal community composition. Metal contamination did significantly alter the composition of the stream meiofaunal assemblages, with certain cyclopoid copepods abundant at high metal concentrations. The meiofauna at sites with high metal concentrations were not significantly less diverse than the meiofauna at sites with low metal concentrations (Burton et al., 2001), highlighting the value of multivariate statistical analysis over the use of univariate statistics.

The development of molecular techniques to analyse the taxonomic composition of *microbial communities* (Liu et al., 2007; Nocker et al., 2007) has greatly opened up the potential to use differences in these communities that might occur in response to anthropogenic contamination. Thus Automated Ribosomal Intergenic Spacer Analysis (ARISA) (Fisher and Triplett, 1999) and Terminal Restriction Fragment Length Polymorphism (T-RFLP) were used to analyse the biofilm bacterial and ciliate protozoan communities at 23 urbanized stream sites in the Auckland region of New Zealand variously impacted by copper, lead and zinc (Ancion et al., 2013). Concentrations of the three metals in biofilms explained 7% of the variation in the bacterial biofilm communities, and 9% of that in ciliate protozoan communities (Ancion et al., 2013).

1.3.6 Biomarkers

The concept of biomarkers was introduced earlier in this review and biomarkers are increasingly being suggested for use in freshwater biological monitoring (Chaumot et al., 2015; Christophe et al., 2015). Here we discuss biomarkers because of their potential use as evidence linking the effects of metal contamination to the community level impacts required by Water Framework Directive legislation.

As defined earlier, a biomarker is a biological response (e.g. a biochemical, cellular, physiological or behavioural variation) that can be measured at the lower levels of biological organization, in tissue or body fluids or at the level of the whole organism (Figure 1.7). Thus a biomarker is a measurable biological response to a raised local bioavailability of a toxicant such as a trace metal, and can be used to assess the ecotoxicological significance of the toxic exposure (Luoma and Rainbow, 2008). Many biomarkers of toxic metal pollution in aquatic habitats have been proposed, assessed and are now being widely employed (Luoma and Rainbow, 2008; Amiard-Triquet et al., 2013). An ideal biomarker in ecotoxicology would be toxin-specific (e.g. d-amino levulinic acid dehydratase for lead exposure, Amiard and Amiard-Triquet, 2013) but most biomarkers lack specificity, being responsive to more than one stressor and indicative of the general health status of an organism. Nevertheless, these biomarkers are of considerable value as a measure of exposure in ecotoxicological assessment. A biomarker is of particular use if it is contaminant-sensitive (and therefore detectable at low levels of biological organization: Figure 1.8), and links (almost certainly correlational by necessity, e.g. Figure 1.9) can be established between its detection in exposed organisms in the field and consequent ecotoxicological effects at higher levels of biological organization, ultimately up to the population, community and eventually ecosystem (Luoma and Rainbow, 2008; Amiard-Triquet and Amiard, 2013a; Moore et al., 2013).

The hierarchy of levels of biological organization defined for biomarkers might typically consist of molecular, biochemical, cellular, physiological, individual organism, population, and community levels, with inevitably some degree of overlap between these levels.

At the molecular biology level toxins may bind to DNA and interrupt normal metabolic functioning, with the ultimate ecotoxicological effect being carcinogenesis (the development of tumours), heritable mutations and teratogenesis (the malformation of embryos), expressed at higher levels of organisation (Luoma and Rainbow, 2008). A first stage in the action of a genotoxin is the formation of an adduct, a covalent bond between toxicant and

DNA, and/or a highly reactive free radical may be generated. DNA strand breakages may be reparable to a point but ultimately damage to chromosomes and cell division can occur. The comet assay is a commonly used biomarker of the early stages of damage to DNA, for it uses electrophoresis to identify the presence of broken DNA fragments in individual cells of exposed organisms (Luoma and Rainbow, 2008; Vasseur et al., 2013).

Biochemical level biomarkers first employed include the metal-binding protein metallothionein and general biochemical stress responses (Roméo and Giambérini, 2013), although, with modern molecular biological approaches, we are in the era of 'omics' – studies on the genome, the proteome, the transcriptome and the metabolome.

Metallothioneins (MT) are non-enzymatic proteins that are induced by and bind to particular trace metals such as silver, cadmium, copper, mercury and zinc; they are of low molecular weight (12-15 kDa), have a high content of the sulphur-containing amino acid cysteine (providing binding sites for the trace metals), no aromatic amino acids and heat stability (Amiard et al., 2006; Luoma and Rainbow, 2008). MTs play a role in the homeostasis of essential metals like copper and zinc, and in the cellular detoxification of both essential and non-essential metals. The induction and metal-binding properties of metallothioneins, which are widespread in invertebrates and vertebrates, initially generated a great deal of excitement in their potential for use as specific biomarkers for toxic metal exposure in the field (Amiard et al., 2006). While there are field examples of the induction of MTs in organisms in contaminated field conditions, MT induction can be variable, for example with species, metal, exposure concentration and with the different MT isoforms (slightly different forms of the same protein resulting from co-occurring slightly different genes) that might be present (Amiard et al., 2006). Moreover, it is now appreciated that MTs can also be induced by other stress factors that are not related to metal physiology, such as anoxia, handling, starvation, freezing and the presence of antibiotics, herbicides or vitamins (Amiard et al., 2006; Luoma and Rainbow, 2008). Furthermore, like other proteins, MTs are turned over in the cell (a process usually involving breakdown in lysosomes (autophagic vesicles in cells), and an increased rate of MT synthesis on metal exposure may be associated with an increased rate of MT turnover but not an increased concentration of MT which is the typical measure to be used as a biomarker (Amiard et al., 2006; Luoma and Rainbow, 2008). Thus metallothioneins are no longer considered to be the specific biomarkers of toxic metal exposure once hoped, but any observed induction of MTs in organisms at sites under investigation for metal ecotoxicology may contribute to an increasing package of relevant field observations to this end.

More general biochemical stress responses that are used as biomarkers include stress proteins and biochemical defences against oxyradicals which can be generated by trace metal exposure as well as by other stressors particularly organic contaminants (Luoma and Rainbow, 2008). Stress proteins make up a set of protein families originally called heat shock proteins, but now known to be induced by exposure to many stressors including, for example, organic compounds, ultraviolet radiation, salinity change as well as toxic metals. Antioxidant defences against oxyradicals (which may for example damage DNA) include primary antioxidant enzymes like superoxide dismutase and catalase which have been measured as biomarkers for some time (Roméo et al., 2009). Other biomarkers include concentrations of malondialdehyde, a breakdown product of lipid peroxidation caused by oxyradicals, and glutathione, an oxyradical scavenger (Roméo et al., 2009). A widely used biomarker is Total Oxyradical Scavenging Capacity (TOSC), an integrated measure of antioxidant defence, to which toxic metal exposure will contribute (Regoli, 2000; Regoli et al., 2011). Again these more general biochemical stress biomarkers may not be specific to toxic metal exposure but they can contribute to a general battery of biomarkers assessing the general health status of an organism being investigated for potential ecotoxicological stress.

Modern molecular biology addresses the whole genome and its expression, as opposed to specific genes and their associated proteins as analysed in the past. In this age of 'omics', genomics is the study of the genome, the entirety of an organism's hereditary information including both genes and non-coding regions; proteomics is the study of the full set of proteins (the proteome) encoded by the genome; transcriptomics is the study of the transcriptome, the set of all RNA molecules produced, reflecting the genes actively being expressed at a particular time and varying with environmental conditions, including exposure to raised trace metal bioavailability; metabolomics studies the metabolome, the complete set of small molecule metabolites found in a biological sample, which is again variable under different contaminant exposure conditions. Transcriptomic and metabolomic studies are typically still at the laboratory stage of investigation, as for example in the cases of rainbow trout gill cell culture studies of the expression of genes for MT, zinc transporters and glutathione-S-transferase (Walker et al., 2007, 2008). Nevertheless, transcriptomics and metabolomics do have particular potential for use in biomarker studies in the field, as we try to understand which genes are induced or show reduced transcription and which metabolites are present under conditions of raised trace metal exposure (Luoma and Rainbow, 2008, Gonzalez and Pierron, 2015).

Biomarkers have also been developed at the cytological level. Genotoxic damage referred to above can lead to chromosomal changes visible in cells under the microscope. One common cytological manifestation of such damage is the presence of micronuclei, displaced from the main nucleus of the cell (Vasseur at al., 2013). Michailova et al. (2009) identified aberrations in the structure of salivary gland chromosomes of larvae of the chironomid *Chironomus acidophilus* in the Afon Goch, a small river draining the former copper mining area of Parys Mountain in Anglesey. The Afon Goch is subject to periodic acid run off and has high water concentrations of iron, manganese and zinc, as well as copper.

Other widespread cytological biomarkers of toxic metal pollution in aquatic habitats centre on *lysosomes*. Lysosomes are membrane-delimited organelles in cells containing hydrolytic enzymes that break down structures or molecules that have originated within or outside the cell. Lysosomes occur in nearly all cells of eukaryotic organisms and they serve to break down for recycling redundant or damaged cell organelles and proteins (including MT: Moore et al., 2006, 2013). Increased functioning of the lysosomal system is a sign of general stress. Thus there are several identified responses of lysosomes to stress that have potential as biomarkers, including changes in lysosomal size and number in particular cells, production of lipofuscin and destabilisation of the lysosomal membrane (Moore et al., 2006, 2013). Lipofuscin is a pigment that is the end product of lipid peroxidation of cell components brought about by reactive oxygen species, and its increased production in lysosomes indicates increased lysosomal turnover activity, as might be caused by exposure to both toxic metals and organic contaminants (Figure 1.12). Lipofuscin granules may indeed contribute to the detoxification of excess cellular trace metals, for example as part of the turnover of MT. The functional stability of the lysosome membrane (and hence its permeability) changes with degree of exposure to contaminants including toxic metals, and the assessment of lysosomal stability based on the dye neutral red (Neutral Red Retention NNR) has become widely used in ecotoxicology because it is both simple and quantitative (Svendsen et al., 2004; Moore et al., 2006, 2013). In practice, blood cells from the animal of interest are applied to glass slides, treated with neutral red, and the time measured for the leakage of the dye from the lysosomes into the cytosol of the cell (Luoma and Rainbow, 2008). Measurement of lysosomal stability is a sensitive, low organisational level biomarker and excitingly its quantification has been correlated with other biomarkers at other levels of biological organisation, including TOSC and Scope for Growth (Figure 1.9: Moore et al., 2006, 2013).

Biomarkers at the biological organisation level of the organism can be morphological or physiological. There are limited records of morphological abnormalities of organisms

resulting from field exposure to high availabilities of toxic metals, and these usually concern diatoms or chironomid midge larvae. Falasco et al. (2009) have produced a wide-ranging review of abnormally developed (teratological) forms of diatoms in the field, and exposure to toxic metals figures strongly as a cause of these morphological abnormalities. There is a significant correlation between valve size reduction and trace metal contamination, and abnormal development appears to be more common as valves decrease in size. Particular diatom species – e.g. *Achnanthidium minutissimum*, *Fragilaria gracilis*, *F. rumpens*, *F. crotonensis* and *F. tenera* – show enhanced abnormal morphologies (e.g. valve distortion and abnormal valve striation) in response to trace metal (cadmium, copper, mercury, zinc) bioavailabilities at metal-contaminated sites, for example in Lake Orta (Italy), the Riou Mort (France) and the Rocky Mountain River (Colorado, USA) (Falasco et al., 2009).

In the case of chironomid larvae, deformities of the mouthparts have long been known to occur in midge larvae living in contaminated sediments (e.g. Di Veroli et al., 2014), and cause/effect relationships between the presence of such deformities and equivalent sediment concentrations of metals (particularly Cu) have been demonstrated in the laboratory (see Martinez et al., 2003). Mouthpart deformities of *Chironomus tentans* larvae include fused teeth, split teeth, missing teeth, extra teeth and abnormally shaped teeth on the mandible, with apparently different effects being associated with different metal exposures (Martinez et al., 2003). De Pauw and Heylen (2001) used a measure of Percentage Mentum Deformities (Warwick, 1988) in unspecified *Chironomus* larvae as a contributing index in a biological assessment of the environmental quality of freshwater sediments in Flanders. It appears that chironomid mouthpart deformities may offer relevant contributing evidence in any ecotoxicological study of metal-rich stream sediments.

Physiological biomarkers include physiological condition indices (particularly for fish – Christophe et al., 2015), growth rates, feeding rates and a popular measure 'Scope for Growth' (SFG). The latter two at least, however, involve collection of animals for subsequent laboratory experimentation, reducing their ease of applicability in any field study.

As regards growth rates, Faria et al. (2007, 2008) measured the growth rates *in situ* of *Chironomus riparius* larvae in mining-affected streams in Portugal, showing inhibition of growth rate (body length increase) in metal-contaminated streams. Incidentally, the results of Faria et al. (2007) confirmed that the midge larvae were more affected by metals entering the body through ingested sediment than by metals dissolved in the water column. Brown trout (*Salmo trutta*) in the metal-mining affected Clark Fork River in Montana, USA were smaller than fish of the same age in carefully chosen reference sites (Tohtz, 1992).

Feeding rates feature strongly in the literature of the effects of trace metal contaminants in freshwater, for example in the case of freshwater gammarid crustaceans, whether standing alone (e.g. Taylor et al., 1993) or as part of the calculation of SFG (Maltby et al., 1990a, b, Chaumot et al., 2015). Maltby et al. (2002) found inhibition of the *in situ* feeding rates of *Gammarus pulex* downstream of point source effluent discharges in UK rivers, and Dedourge-Geffard et al. (2009) showed reduced feeding rate of *Gammarus fossarum* at metal-contaminated sites in the Amous River, France, receiving drainage from a former lead-zinc mine.

SFG integrates different physiological measures in a calculation of the energy balance of an organism (Luoma and Rainbow, 2008). SFG is an estimate of the surplus energy available to an animal for growth and reproduction, calculated from the difference between energy assimilated from food and the energy used in respiration. SFG is interpreted to decrease when energy is required to cope with the extra physiological cost of handling (detoxifying or excreting) high amounts of toxins taken up in contaminated environments. Thus Maltby et al. (1990a, b) showed that the freshwater amphipod *Gammarus pulex* had reduced SFG when exposed to 3 mg L^{-1} zinc. SFG is a very useful integrated measure of how well an animal is

coping with a high bioavailability of a contaminant such as a toxic metal, but it is not easy to measure, requiring rapid access to laboratory facilities to measure such physiological parameters as feeding rate, assimilation efficiency and respiration rate. Nevertheless a correlational link has been established between lysosomal stability and SFG (in this case in mussels *Mytilus edulis* in the marine environment (Figure 1.12: Moore et al., 2006, 2013), again linking biomarkers at different levels of biological organisation.

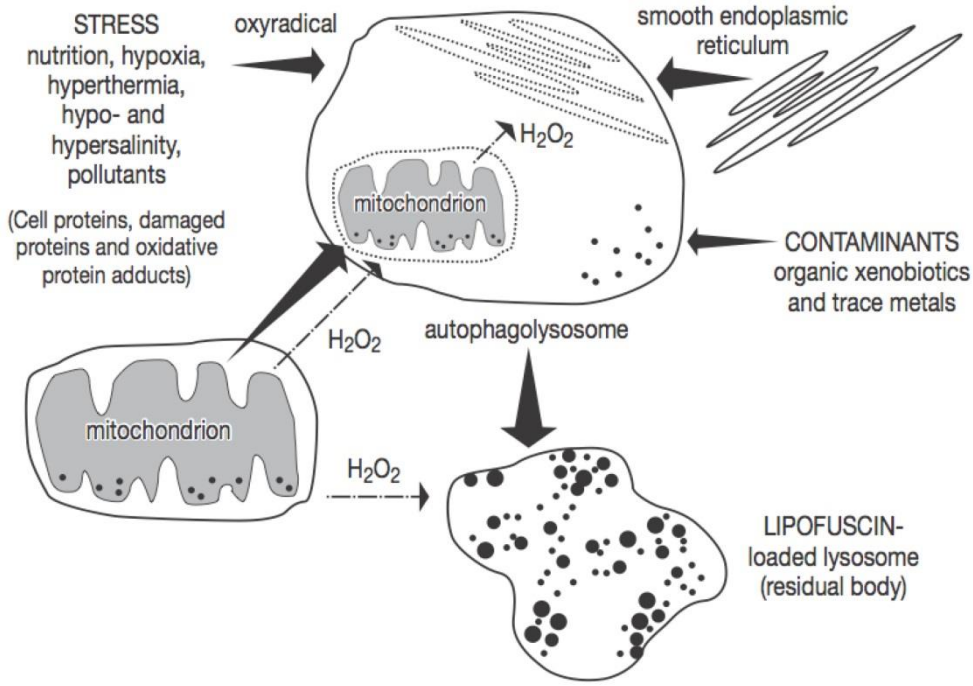


Figure 1.12. The formation and accumulation of lipofuscin in lysosomes (after Moore et al., 2006; Luoma and Rainbow, 2008).

Another integrative response at the organism level that can be used as a biomarker given appropriate observational facilities is the behaviour of an animal, at least those aspects of behaviour affected by toxic exposure (Amiard-Triquet, 2009; Amiard-Triquet and Amiard, 2013b). Behavioural changes at an individual organism level also clearly have the potential to induce knock-on effects at population and community levels (Amiard-Triquet and Amiard, 2013b), as in the case of a reduced swimming capacity of a fish to capture prey and avoid predators (Weis et al., 2001). Furthermore behavioural disturbances originate in biochemical and physiological impairments such as neurotoxicity disrupting the function of sensory systems and endocrine disruption, thereby linking responses at different levels of biological organisation (Amiard-Triquet and Amiard, 2013b). There have been many examples of dissolved trace metals upsetting fish behaviour, particularly in the laboratory, and at concentrations of the order of magnitude of those encountered at contaminated sites in the field (Hansen et al., 1998; Amiard-Triquet and Amiard, 2013b). High trace metal concentrations in sediments and diet can also affect the behaviour of invertebrates and vertebrates (Amiard-Triquet and Amiard, 2013b). Behaviour is an individual response that is clearly linked to biochemical and physiological responses that provide early warning biomarkers, and to population effects resulting from the like of reduced feeding success, reduced longevity and reduced reproductive success (more ecologically relevant biomarkers) (Amiard-Triquet and Amiard, 2013b). Observation of behavioural changes associated with exposure of animals to metal-rich sediments can therefore contribute valuable information to a portfolio of evidence on the ecotoxicological effects of such sediments, but again it would be necessary to use observational (laboratory) facilities after field collection of affected individuals.

Ecotoxicological effects observed at the population level have clear ecological significance for the biota in a contaminated habitat. Such effects might be on the numbers of individuals present, population age structure, reproductive rates or recruitment rates. The selection of metal-tolerant strains is another population-level effect dealt with in more detail below. Laboratory-based studies have shown that metal exposure can affect population parameters such as rate of population increase (e.g. the freshwater amphipod *Gammarus pulex*, Maund et al., 1992) or life-table parameters like percentage hatch, juvenile survival, fecundity, time to maturity, etc., in the case of the freshwater gastropod mollusc *Biomphalaria glabrata* (Salice and Miller, 2003). Field-based evidence is less forthcoming. Nevertheless, Schmidt et al. (2013) provide evidence on the emergence of adult insects of many families from aquatic larvae along a gradient of metal contamination in streams in the Rocky Mountains in Colorado, including the Colorado Mineral Belt that has been mined for the past 150 years. Larval densities decreased in non-linear fashion with water metal concentrations with a precipitous fall above a threshold of combined modelled metal bioavailabilities. Adult emergence also showed a non-linear threshold response with a steeper decline below this same threshold and a more modest decline thereafter (Schmidt et al., 2013). Schmidt et al. (2013) concluded that adult emergence (a population level parameter) was a more sensitive indicator of the effect of low metal concentrations on aquatic insect communities compared with larval density, presumably because successful emergence is limited by a combination of larval survival and other factors. Thus, the ecotoxicological effects of metal exposure on the populations of the insect larvae were not all manifested until later in life, during metamorphosis and emergence.

Ecotoxicological effects of exposure to contaminants that are apparent at the population level have obvious implications for local community structure, via such effects as loss of species and/or abundances of individuals of each species present. Any resulting changes to community structure are clearly acting at the highest level of biological organisation recognised in the hierarchy of biomarkers.

The application of biomarkers in the ecotoxicological assessment of contaminants in freshwater lacks behind that in coastal environments, but there are specific projects in Europe actively pursuing this topic (Collier et al., 2013). For example, in France, INERIS (National Institute for Industrial Environment and Risks) has been developing biomarkers in several freshwater fish species to assess the effects of contaminants with the potential to be used in environmental regulation within the Water Framework Directive (Sanchez and Porcher, 2009). These biomarkers have included biomarkers of oxidative stress, neurotoxicity, and reproductive and immunological disruption. Allan et al. (2006) conclude that biomarkers have shown potential as sensitive methods for the detection of pollution and suggest that they could become important tools within the context of the WFD as part of the evidence linking pollutants to community level impacts.

1.3.7 Tolerance as indicator of significant ecotoxicological selective pressure

The presence of a metal-tolerant population of an organism in a particular habitat is evidence that local bioavailabilities of that toxic metal are of ecotoxicological significance, clearly to that species but potentially also to other members of the local biota (Luoma, 1977). Thus, the local raised metal bioavailability has acted as a selection pressure, selecting for biochemical and physiological traits that are the most metal-tolerant in the gene pool of a local population of organisms, leading then to the establishment of a local metal-tolerant population (Luoma, 1977; Klerks and Weis, 1987).
It needs to be restated here that, in this review, the term metal-tolerant is used to refer to a particular metal-exposed population, whether or not that tolerance is inheritable after selection over several generations or has been derived by physiological acclimation and is restricted to the one exposed generation (Amiard-Triquet et al., 2011). The term metal-resistant was used earlier to refer to species which survive relatively well generally in

conditions of metal contamination, without specific physiological acclimation or genetically based adaptation to the high metal bioavailability in a particular local habitat

The presence of metal-tolerant populations has long been recorded from UK streams affected by mining activities (Kelly, 1988). Harding and Whitton (1976) showed that populations of the alga *Stigeoclonium tenue* abundant in zinc-contaminated streams in the Northern Pennines showed increased tolerance to exposure to dissolved zinc over populations in streams with lower zinc levels. Say et al. (1977) added the algae *Hormidium rivulare* and *H. flaccidum* to the list of species with zinc-tolerant populations in north-east England. Foster (1977) reported on a copper-tolerant population of another alga, the green alga *Chlorella vulgaris*, from the River Hayle draining disused copper mines in Cornwall, the relevant physiological mechanism of tolerance being copper exclusion. In a follow up study Foster (1982) identified 19 metal-tolerant strains of green algae (Chlorophyta) from the River Hayle and the nearby lead-rich River Gannel, with several of the copper-tolerant strains from the Hayle also showing a co-tolerance to lead.

In a parallel study of the freshwater isopod crustacean *Asellus meridianus* in these rivers, Brown (1977) also demonstrated the presence of tolerance and co-tolerance (tolerance to more than one metal in the same population). The Hayle isopod population was tolerant to both copper and lead, although the River Hayle contains low lead and high copper concentrations; the Gannel population was tolerant only to lead, the lead-rich River Gannel lacking high copper concentrations (Brown 1977). Thus, it appears that the mechanism for copper tolerance in the River Hayle population has simultaneously achieved lead tolerance in the absence of raised lead bioavailability, but that the mechanism for lead tolerance in the River Gannel population is not associated with simultaneous copper tolerance in the absence of raised copper bioavailability.

The argument that the evolution of metal-tolerance is a manifestation that local metal exposure is of ecotoxicological significance can be extended to the structure of the local biotic community. Blanck et al. (1988) proposed that the selection pressure associated with an ecotoxicologically significant toxicant bioavailability will lead to an increased average tolerance to that toxicant among all species within that local community. Such *Pollution-Induced Community Tolerance (PICT)* is therefore a potential ecotoxicological tool to assess the effects of a toxicant on communities (Blanck and Wangberg, 1988; Blanck et al., 1988; Clements and Newman, 2002; Luoma and Rainbow, 2008; Schmitt-Jansen et al., 2008).

PICT is tested by comparing communities collected from contaminated and reference sites to contaminant exposure under controlled conditions, and increased community tolerance that results from the elimination of more sensitive species is considered to be strong evidence that community restructuring has been caused by the contaminant (Clements and Newman, 2002; Clements and Rohr, 2009). The need to carry out experiments to measure the difference in tolerance between communities does constrain the application of PICT as an assessment tool, although PICT has been tested in several different communities beyond the marine periphyton community on which the PICT hypothesis was first developed (Blanck and Wangberg, 1988; Clements and Newman, 2002). Such communities include lentic periphyton, lentic phytoplankton, marine phytoplankton, lotic microalgae, estuarine nematodes, and freshwater and coastal macroinvertebrates, and the contaminants concerned include the trace metals arsenic, cadmium, copper and zinc (Clements and Newman, 2002; Luoma and Rainbow, 2008; Clements and Rohr, 2009). Among the assumptions behind PICT is that the communities most likely to be suitable for PICT assessment are those that show a large amount of variation in sensitivity among species (Clements and Newman, 2002), and PICT may prove particularly suitable for comparison of microbial populations collected from different field sites (Tlili and Montuelle, 2011; Virsek et al., 2013).

An alternative approach to testing PICT on individual community samples is to establish species sensitivities *a priori*, and use these to estimate the community level tolerance. This approach works best when field-based responses to the stressor are used to establish sensitivity, as this avoids the complications of extrapolating from the laboratory to the field.

1.3.8 Metal ecotoxicology and ecosystem function

This review has focussed on how a chemical stressor such as the trace metal contamination of sediments affects community structure in freshwater streams, highlighting the observed community shift from sensitive to resistant species as the former are lost. Another approach is to consider the ecotoxicological effects of stressors on ecosystem processes (Clements and Rohr, 2009). Not all species are equally important for the functioning of an ecosystem. Where functional redundancy occurs the loss of some species may not affect ecosystem processes (Reiss et al. 2009). A linear relationship between species richness and ecosystem function would occur where there is no functional redundancy and equal importance of all species present, whereas the opposite would result in an abrupt decrease in ecosystem function at a critical threshold of species loss (Clements and Rohr, 2009). These 'linear' and 'rivet' models have different ecotoxicological implications. In the linear model, interspecific differences in contaminant sensitivity will have relatively little effect on ecosystem processes, while the rivet model predicts that the elimination of any species could result in the abrupt loss of ecosystem function (Clements and Rohr, 2009).

Several studies have taken an ecosystem function approach when investigating the ecotoxicological effect of metal pollution in streams and rivers. For example, Schultheis et al. (1997) showed that copper pollution in East Prong Creek, Virginia, USA, derived from an abandoned pyrite mine, reduced taxonomic richness and abundance of benthic macroinvertebrates. Leaf decomposition rates were also decreased at the affected sites, apparently by the interruption by copper pollution of the action of shredders processing leaf material in the streams (Schultheis et al., 1997). Maltby et al. (2002) similarly reported that the decrease in rates of feeding (leaf shredding) by *Gammarus pulex* downstream of point source discharges in UK rivers was correlated with a decrease in local leaf decomposition rates. Furthermore, Carlisle and Clements (2005) showed that the secondary production of macroinvertebrate shredders was negatively correlated with dissolved zinc concentrations in Colorado streams affected by mining, with associated declines in leaf litter breakdown rates and microbial respiration. Carlisle and Clements (2005) concluded that some shredder species contribute disproportionately to leaf litter breakdown, and that the functionally dominant taxon (the stonefly *Taenionema pallidum*) was also the most sensitive to metal contamination. There was no functional redundancy in leaf litter breakdown in the study streams, and leaf litter breakdown was highly sensitive to metal contaminant-induced alterations in community structure (Carlisle and Clements, 2005). Carlisle and Clements (2005), therefore, argue for the necessity to measure ecosystem function as well as community structure in assessments of the ecotoxicological effects of anthropogenic contaminants in stressed ecosystems. Whilst conceptually appealing, the use of measures of ecosystem function to detect pollution problems is difficult, as attribution of an observed change to a specific cause is not straightforward; frequently there are multiple different potential mechanisms which could result in the same observed change.

In a review of freshwater macroinvertebrate trait-based community descriptors, Menezes et al. (2010) highlighted that the ecosystem functional trait approach is one of the most promising tools emerging for the biological monitoring of freshwater ecosystems, but that further research is still required to develop a broad unified monitoring tool, not least for the detection of specific stressor impacts.

As for community structure analysis, there is tremendous scope for the further expansion of genomic and metagenomic approaches to investigate the ecosystem functional processes at the microbial level, involving such indices as the Average Metabolism Response (AMR) and Community Metabolism Diversity (CMD).

1.3.9 Separating effects of metal-rich sediments from other effects of mining

In the real world, any assessment of the ecotoxicological effects of mining-associated metal-rich sediments in streams will be confounded by the presence of other stressors with potential detrimental effects on biotic communities. By definition one such stressor will be the very presence of the sediment itself.

a Sediment

The accumulation of excess fine sediment, even in the absence of any significant contaminant loading, will itself cause changes in the expected biotic communities of streams, not least in the case of benthic macroinvertebrates, be it by direct effects such as burial, clogging and associated oxygen availability or by indirect effects like effects on habitat or food availability (Jones et al., 2012b; Extence et al., 2013). Hence, the UK TAG has concluded that sedimentation has the potential to threaten such ecological status in riverine systems (Collins and Anthony, 2008; Collins et al., 2009). Extence et al. (2013) developed a new index, the *Proportion of Sediment-sensitive Invertebrates (PSI)*, at family PSI (F) and species PSI (S) levels, to assess the quantity of inert fine sediment accumulation at a site through analysis of the macroinvertebrate community. Following the principles used to develop LIFE, expert opinion was used to assign species and families of British benthic macroinvertebrates to one of four Fine Sediment Sensitivity Ratings (FSSR) after a literature review and consideration of their biological traits (Extence et al., 2013). Taxa in each rating are scored according to their abundance, and the PSI calculated from a ratio of the summated scores of sediment-sensitive taxa to the summated scores of all taxa (Extence et al., 2013). A different approach, based on empirical field data rather than expert opinion, was used by Collins et al. in Defra project WQ0128 (Collins et al. 2012a) to determine the sensitivity of invertebrate species to fine sediment pressure. Here, partial ordination analysis was used to rank species against measured deposited fine sediment and, thus develop an macroinvertebrate index to detect fine sediment pressure, CoFSI (Combined Fine Sediment Index): a combined index had to be produced because it was found that invertebrate species responded independently to the organic and inorganic fractions of fine sediment (Murphy et al., 2014). The CoFSI index has been linked to land use models that predict the fine sediment pressure from agricultural activity, ASPIRE (Agricultural Sediment Pressure and Impact on River Ecology), for use in catchment management (Collins et al. 2013). Beyond the benthic macroinvertebrate community, fine sediment in rivers will also impact on fish (Kemp et al., 2011), macrophytes (Jones et al., 2012a) and diatoms (Jones et al., 2014).

In a synthesis of the literature on the effects of sediment deposition on benthic macroinvertebrates and fish in rivers, Collins et al. (2011) briefly review the key factors controlling the impacts of sediment on freshwater biota, and in light of the needs of the WFD, review international approaches in setting sediment targets for rivers to protect or enhance their ecological status. A feature of existing international guidelines relating to critical sediment concentration thresholds is that they are founded on a direct (linear) relation between sediment concentration and ecological impact (Collins et al., 2011). As exemplified several times in this review, such a simple linear model is undoubtedly an oversimplification, and there is a pressing need for revised sediment targets in rivers to inform river catchment water policy with toolkits founded on the coupling of sediment pressures and biological response (Collins et al., 2011).

b Other co-varying stressors

There will be other co-varying stressors potentially present when carrying an ecotoxicological assessment of the effect of metal-rich sediments in streams affected by mining. These will need to be recognised and evaluated in any attempt to identify those

ecotoxicological effects specifically down to the presence of toxic-metal contaminated sediments.

Such physical stressors will include the composition of the stream bed and the presence or absence of manmade channelization and consequent changes to flow regimes. Even these stressors will covary, as for example the composition of the stream bed and sediment loading.

Chemical stressors will include levels of dissolved trace metals and their dissolved bioavailabilities, linked in turn to pH (as in AMD) which will have a direct effect on biota even in the absence of high metal bioavailabilities (Gerhardt, 1993; Davy-Bowker et al., 2005). The effects of increased acidity due to mine drainage may have as much impact on biota as the toxic effects of metals (Hogsden et al., 2013). Dissolved organic matter (DOM), perhaps derived from the sewage of mining communities, can affect biota directly as well as indirectly by chelating dissolved metals and reducing their dissolved bioavailabilities (Luoma and Rainbow, 2008). Levels of organic matter in the sediment will also affect the community structure of macroinvertebrates, as seen in the use of many of the biotic indices discussed above. Urban development around mines (to house workers) can lead to increased sewage inputs in the vicinity of the mine. Other anthropogenic contaminants like PAHs or organochlorine compounds are likely to be introduced into river systems on passage downstream, although arguably these might be at relatively low levels in upland streams draining orefields with abandoned mines.

1.4 Explanation and prediction of metal ecotoxicological effects

Under the Water Framework Directive (European Parliament, 2000), the UK is obliged to achieve Good water status (Good chemical status and Good ecological status) of water bodies, including mining-affected streams. Ambient environmental standards are used in the classification of water bodies on the basis of chemical contamination particularly of priority hazardous substances, and these standards (Environmental Quality Guidelines EQG for both water and sediments) are set to prevent damage from contaminants like trace metals. As for good ecological status, the WFD places a legal obligation on EU nations to use biota to assess the ecological quality of a water body, and this is typically achieved largely through community composition analysis. The WFD defines the biological quality elements to be assessed as benthic macroinvertebrates, phytoplankton, phytobenthos (macrophytes and benthic algae), and fish, depending on the type of water body. Phytoplankton are not relevant to rivers, but benthic macroinvertebrates, phytobenthos (including algae, bryophytes, angiosperms) and fish are worthy of attention in this context.

To explain and predict how metal-rich sediments in mining-affected streams exert ecotoxicological effects on the local biota, there is a need to understand how metal toxicity/bioavailability causes effects at the community level. Thus we need to demonstrate what ecological effects are caused by metals, at what concentration and under what conditions – i.e. how much metal is a problem, with an evidence base behind the choice of measure.

Can we use a relatively simple biological assay of local metal bioavailability to interpret analysis of community composition? Subsequently we can then address the question “Can we determine where (and how much) restoration effort is needed by understanding the link between bioavailability and community ecological effects?”

In this section we introduce existing methods used to address these questions and attempt to draw summarised conclusions on a resource-efficient way forward.

1.4.1 Laboratory testing - Bioassays

Historically environmental regulations have drawn on a database of laboratory-based acute toxicity testing using high dissolved concentrations of toxic metals. Such acute toxicity tests have served to rank metals in order of (dissolved) toxicity and to rank species in order of sensitivity to such dissolved toxic metals. Other fundamental findings from such toxicity testing are that metals are toxic at what appear to be low dissolved concentrations; the lethal concentrations are repeatable if determined under the same conditions but vary with test conditions; lethal concentrations vary both among metals and among species (Luoma and Rainbow, 2008).

The classic toxicity test is the 96 hour acute (dissolved) toxicity test with death as the endpoint, aiming to provide an estimate of the concentration that will kill 50% of the population – the LC_{50}, where LC refers to Lethal Concentration. If the endpoint is a sub-lethal effect, then the test will provide an EC_{50}, EC signifying an Effect Concentration. Such testing will also provide information on the highest concentration to cause no toxic effect – the No Observed Effect Concentration (NOEC), and the lowest concentration to cause an effect – the Lowest Observable Effect Concentration (LOEC).

Traditional single species toxicity testing has been extended to cover more sensitive endpoints than lethality with longer duration tests at lower exposure concentrations to detect sub-lethal effects; a variety of life history stages was tested; test methods were extended to sediments; and multi-species testing was developed (Luoma and Rainbow, 2008). Such tests do have the advantage of providing to environmental regulators with single numbers to define toxicity, and it is typical to use chronic toxicity data to derive regulatory thresholds, rather than acute data.

We now, however, appreciate the limitations in extrapolating the results of such toxicity testing to real field situations (Luoma and Rainbow, 2008). Toxicological responses derived under controlled laboratory conditions and continuous exposure, whilst defining absolute limits, are likely to be over-prescriptive for variable natural conditions, where spatial patchiness and behaviour of organisms can influence exposure. This disconnect between toxicity tests and field responses is not restricted to metals; similar difficulties in interpretation of toxicity tests have been noted for organic pollution (Jones et al., 2008). Furthermore, animals obtain a significant (often majority) source of metals from the diet, and it is no longer accepted that only metals taken up from solution are ecotoxicologically significant (Croisetière et al., 2006; Cain et al., 2011). This is particularly true at lower dissolved exposures in the field and in the case of sediment-ingesting deposit-feeding animals. Thus, a single dissolved metal concentration derived in the laboratory has little direct relevance to the field, particularly given the variation in physicochemical conditions among contaminated sites, which subsequently will influence the bioavailabilities of the dissolved metals (Luoma and Rainbow, 2008). Similar geochemical differences will occur between sediments tested in the laboratory and sediments in the field, affecting the local exchange of sediment-associated metals with pore water and the water column, and more importantly the trophic availability of metals to sediment-ingesting infauna like oligochaete worms. Biology intervenes also – different organisms in the field will show different relative importance of different metal uptake routes, causing interspecific differences in ecotoxicological responses to the suite of bioavailable metal sources active in the field. This feature is particularly important if inappropriate species have been used in the original laboratory toxicity testing, as in the (at one time common) exposure of a planktonic swimmer to a metal-rich sediment. Given the enormous diversity of responses to metal exposure among fauna and flora, it is not practical for all species to be tested. Time scales of laboratory and field exposures are inevitably different, with most toxicity tests using exposure times of less than the generation time of the test organism as is the case in nature.

These limitations are well recognised now for metals and other pollutants (e.g. Jones et al. 2008), and systematic adjustments (application factors) are typically made to laboratory-derived toxicity measures to take into account potential underestimations of toxicity, and chemical test conditions are set for worst possible cases (Cairns and Mount, 1990; Luoma et al., 2010).

Decades of toxicity testing have allowed databases for many metals to grow and include many species. *Species Sensitivity Distributions (SSD)* can provide evidence on the dissolved metal concentration that would have no ecotoxicological effect (NOEC) on, for example, 95% of species, the percentage defined by the US Environmental Protection Agency EPA to be used in protecting aquatic communities (Luoma and Rainbow, 2008). SSD plot the NOEC of a metal for different species against the cumulative rank of the value among species (Figure 1.13: Brix et al., 2001), and risk is estimated as the percentage of species expected to be affected at any dissolved concentration. The SSD method is the preferred approach to setting Environmental Quality Standards in Europe and the UK.

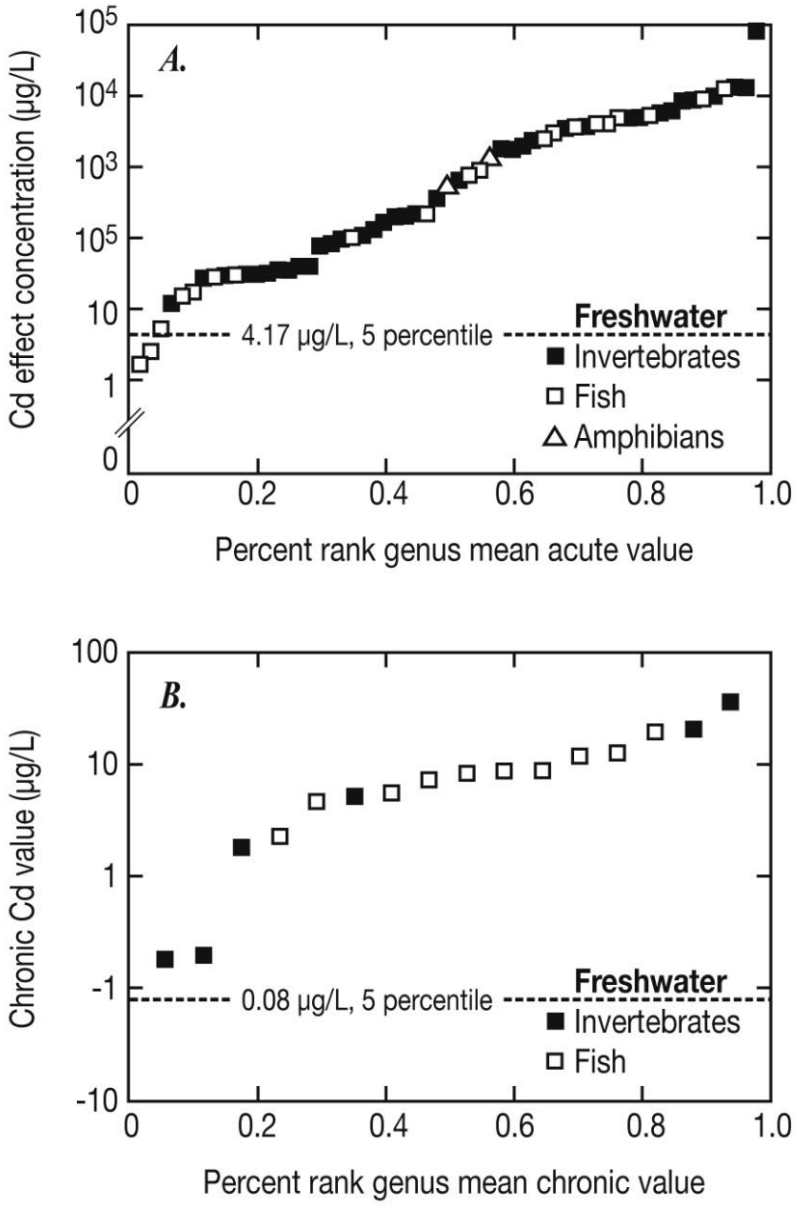


Figure 1.13. Species Sensitivity Distributions (SSD) of:

A. Concentrations of cadmium causing acute toxicity ranked for different genera of invertebrates, fish and amphibians. This SSD can be used to derive a freshwater final acute value for cadmium – the cadmium concentration at which toxicity was observed for the most sensitive 5% of taxa (4.17 µg/L at 50 mg/L hardness).

B. SSD based on chronic toxicity tests. Sensitivity at the 5th percentile occurs at 0.08 µg/L at 50 mg/L hardness. (After US EPA, 2001; Luoma and Rainbow, 2008).

The SSD approach can be used in risk assessment through a Predicted Environmental No Effect Concentration (PNEC), which can be estimated by dividing the lowest value or a percentile (usually fifth percentile) by an arbitrary safety factor (usually between 1 and 100, Crane et al., 2007). A Predicted Environmental Concentration (PEC) in the habitat being assessed can be calculated, for example, from emission rates of the contaminant in an effluent and the diluting capacity of the receiving water body, and a risk quotient (PEC/PNEC) derived: a value well below 1 indicates a low risk, while a risk quotient of 1 or above indicates substantial risk.

1.4.2 Extrapolation from laboratory to field

The WFD uses ambient Environmental Quality Standards (EQS) to classify water bodies on the basis of chemical contamination, and these standards are set to prevent damage from contaminants like trace metals. In the UK EQS exist for concentrations in water (Water Quality Criteria WQC or Water Quality Guidelines WQG) but are currently lacking for sediment (Sediment Quality Criteria SQC or Sediment Quality Guidelines SQG). Where there are sufficient data, such criteria typically have their origins in SSD as described above (Schmitt-Jansen et al., 2008), otherwise a deterministic method is used.

EU Water Quality Standards applicable to priority toxic metals have been established for UK freshwaters (Table 1.1a). Similar Water Quality Criteria/Guidelines are available for the USA, Canada (http://ceqg-rcqe.ccme.ca/), and Australia and New Zealand (Table 1.1a).

Given that EQS for dissolved metals in freshwaters were based mainly on laboratory toxicity data with associated uncertainties as to their extrapolation to the field, Crane et al. (2007) analysed a dataset from England and Wales of dissolved metal (cadmium, chromium, copper, iron, nickel, lead and zinc) concentrations and associated benthic invertebrate community metrics. Crane et al. (2007) used piecewise ('broken stick') regression, quantile regression and available data on metal concentrations consistent with good quality status (WFD-UKTAG, 2008c), and showed that proposed dissolved metal EQS available were similar to dissolved metal concentrations in rivers with unimpaired benthic macroinvertebrate assemblages. Use of a combination of laboratory testing corroborated by field-scale data provides EQS with pragmatic value in real contaminated situations.

a In situ and mesocosm toxicity tests

Improvements on laboratory toxicity testing have been the development of *in situ* sediment testing, and the application of microcosms to better reflect field situations.

In situ toxicity testing involves the exposure of test organisms within or on the sediments of the water body of interest, typically by caging organisms in direct contact with intact sediments, better simulating natural exposures (Luoma and Rainbow, 2008). *In situ* bioassays with fish have a long history, but methods for testing macroinvertebrates in sediments have been developed more recently (Burton et al., 2003). *In situ* protocols typically require survival of control organism for 10 to 28 day exposure periods, necessitating the use of test organisms that can survive handling stress and fluctuating field conditions. A challenge then is to distinguish between the effects of the metal contamination of the sediment and the effects of handling or responding to fluctuation in the habitat conditions. Stochastic variability can be more problematic than dynamically stable variability such as seasonal cycles. For example, Olsen et al. (2001) transplanted fourth instar *Chironomus riparius* larvae for 48 hours at 13 uncontaminated river sites across southeast England. Activities of two enzymatic biomarkers varied almost twofold across the sites, with statistically significant differences detectable between sites. Olsen et al. (2001) concluded that biomarker results must be treated with caution because natural variability in responses can occur even in the absence of toxicant exposure.

Mesocosms can be used to test ecotoxicological questions that single species bioassays cannot, such as, for example, secondary ecological effects on communities (Clements and Newman, 2002). Although mesocosm studies can be costly, labour intensive, difficult to control and difficult to replicate, (and therefore rarely used in routine toxicity testing), mesocosms are well suited to testing process questions (Luoma and Rainbow, 2008).

Clements et al. (1988), Kiffney and Clements (1994) and Clements (2004) used multispecies experimental mesocosms to investigate field toxicities of metals in Rocky Mountain streams in the USA. The multispecies assemblages to be tested in the mesocosms were typically collected by placing boxes of stones in uncontaminated streams for 40 days to allow colonisation by local fauna. The boxes were then transferred intact to the laboratory to be submerged in experimental mesocosm streams for 10 days (Luoma and Rainbow, 2008). Results from such experiments established a hierarchy of vulnerability to metal exposure among the local taxa inhabiting streams, in line with relative metal sensitivities discussed above. Thus mayflies and stoneflies showed reduced abundance and species richness at the lowest metal concentrations in the mesocosms, and heptageniid mayflies such as *Epeorus longimanus* were particularly sensitive to the metals, in line with field surveys of metal-contaminated streams (Clements, 2004). Numbers of overall taxa, numbers of mayfly taxa, and summed numbers of mayfly (Ephemeroptera), stonefly (Plecoptera) and caddisfly (Trichoptera) taxa (EPT) showed statistically significant metal concentration – response relationships. The mesocosm experiments could be used to generate EC_{10} values (Effect Concentrations affecting 10% of the population), which correspondingly showed higher values for combined taxa than for single sensitive species like *E. logimanus* (Clements, 2004; Luoma and Rainbow, 2008). The functional endpoints invertebrate drift and community respiration (measured by change in dissolved oxygen) were generally more sensitive to metal exposure than structural measures of the invertebrate community (Clements, 2004).

The mesocosms were also used to investigate the effects of mixtures of metals in comparison to those of single metal exposures, zinc, copper and cadmium in the experiments of Clements (2004). The Cumulative Criterion Unit (CCU) is the additive measure of toxicity used in such cases. The dissolved concentration of each metal is divided by the US EPA chronic criterion value for that metal derived from single species toxicity tests but adjusted for water hardness by formula, and the ratios for each metal are summated to give the CCU (US EPA, 1986; Clements, 2004; Luoma and Rainbow, 2008). Macroinvertebrate responses to a mixture of the three metals zinc, copper and cadmium were generally greater than responses to either zinc alone or to zinc and cadmium (Clements, 2004).

The same mesocosm approach to investigate the effects of metal contamination in New Zealand streams (Hickey and Clements, 1998; Hickey and Golding, 2002) has led to similar conclusions, particularly that abundance and species richness of mayflies are sensitive measures of metal effects in streams. The wide generality of such conclusions offers confidence that biological responses to metal contamination in streams are predictable (Luoma and Rainbow, 2008).

Recent community-level mesocosm experiments by Clements et al. (2013) in Colorado have shown that EC_{50} values, defined as the metal concentrations that reduced abundance of stream insects by 50%, were several orders of magnitude lower than values derived for single species from previous laboratory testing. Clements et al. (2013) hypothesise that the short duration of laboratory toxicity tests and the lack of evaluation of effects on early life history stages are the primary factors behind the production of unrealistically high LC_{50} values in the literature. The mesocosm results of Clements et al. (2013) suggest that US water quality criteria for zinc would protect most aquatic species, but that copper was highly toxic to some species at concentrations near to the published copper water quality criteria.

Although the use of mesocosms as described here still involves only the dissolved concentrations of toxic metal as measures of contaminant input, mesocosms are more representative of field conditions, and the results from mesocosm experiments together with field data can provide more precise estimates of 'safe' metal concentrations than laboratory toxicity tests alone (Clements, 2004). Nevertheless, the extrapolation of the results of any laboratory controlled experiment with, for example, continuous exposure/concentration to the field, where concentrations, environmental conditions and behavioural responses are variable, is not straightforward.

The same CCU approach can be extrapolated to dissolved concentrations of metals measured in the field. Thus Clements et al. (2000) expressed field concentrations of zinc, lead, manganese, iron, copper, cadmium and aluminium in CCU in a study of benthic macroinvertebrate communities in meta-contaminated Colorado mountain streams, and Hirst et al. (2002) quantified variations in dissolved metal concentrations in terms of CCU scores in their study of streams in metal-mining areas of Wales and Cornwall.

1.4.3 Modelling of dissolved bioavailability

The incorporation of a formula to correct an Effects Concentration of a metal for water hardness was mentioned above in the calculation of a CCU. The underlying reason for such intervention is the need to get a better measure of dissolved bioavailable metal concentration than is offered by the total dissolved concentration alone. As defined in the introduction, bioavailability describes a relative measure of that fraction of the total ambient metal that an organism actually takes up when encountering or processing environmental media, summated across all possible sources of metal, including water and food as appropriate (Luoma and Rainbow, 2008). Specifically in the case of dissolved bioavailability, it is now generally recognised that only a fraction of the total dissolved metal is available for uptake across a cell membrane into an organism, i.e. is bioavailable. A good model for the dissolved bioavailable fraction of many trace metals is the free metal ion according to the Free Ion Activity Model FIAM (Campbell, 1995; Luoma and Rainbow, 2008).

The release of the free metal ion in the various chemical equilibria affecting a dissolved metal is very dependent on the physicochemistry of the medium. Dissolved organic matter, especially humic acid HA, tends to complex metal ions, thereby reducing the percentage of free metal ion in the total dissolved metal concentration. Acid conditions often promote the availability of the free metal ion, and the presence of other dissolved metals may also affect the release of the free ion from different forms of organic and inorganic complexation. Thus the same total dissolved metal concentration may have very different contributing concentrations of free metal ions, according to local physicochemical variation. Two media of the same total metal concentration may offer very different dissolved metal bioavailabilities to organisms (Luoma and Rainbow, 2008).

In the absence of analytical techniques to measure the concentrations of all the different forms (chemical species) of most metals dissolved in natural waters, speciation modelling has proved a very successful alternative. Thus the activities of the free metal ion and other chosen low molecular weight metal complexes can be calculated from first chemical principles, essentially by considering the total concentration of dissolved metal, the concentrations of potential ligands (ions or molecules that form a complex with a metal ion), and the stability constants defining the affinity of each ligand to the metal. One of the most successful and commonly used such metal speciation models in freshwaters is WHAM, the Windermere Humic Aqueous Model (Tipping, 1994; Tipping et al. 1998), including very thorough analyses of metal-organic (especially metal-HA) interactions and updated regularly (e.g. Stockdale et al., 2010; Tipping et al., 2011). A tenet of the WHAM model is that the dissolved metal cation binding sites of aquatic invertebrates can be modelled by the

functional groups of humic acid, and WHAM can model how much metal will bind to HA under different physicochemical circumstances. The predictor 'bioavailable' dissolved metal concentrations calculated by WHAM in its different updated guises are usually better predictors of the toxicological effects of dissolved metals than are total dissolved metal concentrations. In Colorado, Iwasaki et al. (2013) found that predictor concentrations of zinc, cadmium and copper derived from the WHAM 7 model of Tipping et al. (2011) provided better estimates of metal effects observed in mesocosm experiments than three other measures – total metal concentrations, free metal ion concentrations and the CCU. Stockdale et al. (2010) developed WHAM into the WHAM-F_{TOX} model to describe the bioavailability and toxicity of proton and metal mixtures to aquatic organisms, by incorporating a toxic potency coefficient for each metal. Stockdale et al. (2014), however, got variable levels of success in applying this model to sites in UK and Norwegian streams, and suggested that the presence of other non-chemical (unmodelled) factors was further repressing species richness at some sites.

Biotic Ligand Models (BLMs) are a theoretical offshoot of the FIAM (Paquin et al., 2002), which predict dissolved metal toxicity on the basis of calculated (modelled) free metal ion activities as affected by the competitive effects of major ions and pH (critically important in freshwaters). BLMs were developed to explain how water chemistry affects the toxicity of dissolved metals, toxic exposure being expressed by the occupancy by the metal of a key (biotic) ligand. In the regulatory arena, BLMs can be used to incorporate site-specific physicochemical conditions, using geochemical modelling to calculate metal speciation (Luoma and Rainbow, 2008). An advantage of the BLM is that it shifts emphasis from the exposure solution to a postulated biological receptor. Although based on chronic exposures to dissolved metals, dietary metal uptake is typically not included and uptake properties have only been characterised for a few species (Luoma and Rainbow, 2008). Schmidt et al. (2010) developed a toxic unit model of additive trace metal toxicity (the Chronic Criterion Accumulation Ratio CCAR) derived from BLM outputs to estimate the toxicity of trace metal mixtures to benthic communities (e.g. Schmidt et al., 2012).

There is no doubt that the predictor concentrations coming from dissolved metal speciation models such as WHAM, WHAM-F_{TOX} and the BLM are superior estimators of dissolved metal bioavailabilities than total metal concentrations. However, it still needs to be remembered that such models concern dissolved metal concentrations and ignore the ecotoxicologically significant role of metal uptake from the diet in the real contaminated world.

Not uncommon in the literature now are studies of dissolved metal bioavailabilities that use passive samplers employing the like of DGT (Diffusive Gradients in Thin Film) technology to model (not measure - in spite of claims otherwise) dissolved metal bioavailabilities. Such passive techniques make assumptions as to the chemical nature of dissolved species of metals that are bioavailable to (all?) organisms, to come up with a so-called measure of dissolved metal bioavailability. Nevertheless, it is a flawed argument to employ a chemical surrogate to model (usually ineffectively) what can be measured directly by the employment of a suitably chosen biomonitor. Yet again, this approach ignores any trophic availability of local metals to resident animals, in spite of the demonstrated ecotoxicological importance of this route of uptake.

1.4.4 Bioaccumulation, biomonitors and identification of ecotoxicological effects

Aquatic invertebrates take up trace metals in relation to the total metal bioavailabilities (e.g. dissolved and dietary) to which they are exposed. They are typically net accumulators of trace metals, the strength of the accumulation after uptake being controlled by the subsequent physiological balance between uptake rates, excretion rates and storage detoxification rates Luoma and Rainbow, 2008). The metal accumulation patterns of aquatic

invertebrates vary between metals and invertebrate species, and will lie on a gradient from weak to strong accumulation. Different invertebrate species have different rates of turnover of metals in the bodies and will achieve different accumulated body metal concentrations under the same metal exposure conditions (Luoma and Rainbow, 2008).

These principles allow the use of aquatic invertebrates as biomonitors of trace metal bioavailabilities, a biomonitor being an organism which accumulates trace metals in its tissues, the accumulated metal concentration of which provides a relative measure of the total amount of metal taken up by all routes by that organism, integrated over a preceding time period (Luoma and Rainbow, 2008). Biomonitors are therefore of great utility in biological monitoring programmes, for their use can identify areas of high metal bioavailability (strictly to that chosen biomonitor, but interpreted more generally) and to identify changes in metal bioavailability over space and time (see Introduction). It must be remembered, however, that a high accumulated body concentration of a metal does not *per se* indicate that the invertebrate (or other organism such as a plant) is suffering an ecotoxicological effect. Such a demonstration requires the application of biomarkers from any level from the molecular to the community.

It is not surprising that the changes in the summated bioaccumulated metal concentration of all invertebrate species in a community will not automatically be a good predictor of altered macroinvertebrate community structure (Beltman et al., 1999). The metal concentration of a pooled invertebrate sample will depend not only on local metal bioavailabilities, but on other factors such as the presence and relative contribution of the different invertebrate species present in the sample (with different associated metal accumulation patterns), life history stage, size, age , etc. (Hare, 1992; Beltman et al. 1999). The community composition will be changed on metal exposure in ways that will inevitably affect the total metal bioaccumulation ability of the community (Beltman et al., 1999).

All is not lost, however. The key to the use of biomonitors and metal bioaccumulation in ecotoxicological studies is to interpret accumulated metal concentrations at the species level. Thus, it is well established that the use of individual species as biomonitors provides key information on differences in metal bioavailabilities over space and/or time (Luoma and Rainbow, 2008). Biomonitoring of a water system may make use of the *in situ* fauna, as in the case of copper in the caddisfly *Plectrocnemia conspersa* along a stream system in Cornwall (Gower and Darlington, 1990), or baetid mayflies and the amphipod *Gammarus fossarum* in metal-rich streams in Poland (Fiałkowski et al., 2003a, b). Alternatively, members of a control population of invertebrates may be deployed at different sites for a time period before analysis (e.g. the amphipod crustacean *Hyalella azteca* by Couillard et al. (2008) in mining-affected river systems in Quebec).

As discussed earlier, it is not possible to correlate a specific total accumulated body concentration of an aquatic invertebrate in the field with the onset of toxic effects in that individual, there being no critical (lethal) total body concentration of a metal in an invertebrate using (temporary or permanent) storage detoxification (Luoma and Rainbow., 2008; Casado-Martinez et al., 2010a; Adams et al., 2010; Rainbow and Luoma, 2011b). So there is no specific ecotoxicological information in a bioaccumulated metal concentration relevant to the biomonitor itself. Studies are now showing, however, that it is possible to calibrate accumulated metal concentrations in specific biomonitors against ecotoxicological changes occurring in the local biological community, typically the benthic macroinvertebrate community (Luoma et al., 2010; Rainbow et al., 2012). Luoma et al. (2010) proposed that there will be a correlation between the bioaccumulated metal concentrations in a relatively metal-hardy biomonitor and ecotoxicological changes affecting more metal-sensitive members of the local benthic macroinvertebrate community. Specifically Luoma et al. (2010) addressed this hypothesis in terms of the bioaccumulation of copper in larvae of species of the caddisfly *Hydropsyche*, and the presence and abundance of ephemerellid and

heptageniid mayflies at sites in the Clark Fork River system, Montana, USA. Luoma et al. (2010) indeed were able to show that the bioaccumulated copper concentrations in *Hydropsyche* could be calibrated against metal-specific ecotoxicological responses in the number of species and abundance of mayflies, particularly of ephemerellid and heptageniid mayflies. Rainbow et al. (2012) addressed the question whether the proposed hypothesis of Luoma et al. (2010) would hold in another mine-affected catchment system, in fact on another continent. Rainbow et al. (2012), therefore, specifically asked whether the bioaccumulated concentrations of toxic metals in the caddisfly *Hydropsyche siltalai* (as indicators of metal bioavailabilities) could be calibrated against ecotoxicological responses of mayfly larvae in metal contaminated streams of Cornwall. As in the Clark Fork system, Rainbow et al. (2012) were able to answer in the positive. Mayfly larvae were always sparse where metal bioavailabilities, hence bioaccumulated metal concentrations in the caddisflies, were high, and were abundant where metal bioavailabilities were low, a pattern particularly evident when the combined abundance of ephemerellid and heptageniid mayflies was the response variable (Rainbow et al., 2012). Furthermore Rainbow et al. (2012) were able to identify threshold bioaccumulated concentrations in *H. siltalai* (especially of Cu and As, the apparent major ecotoxicological metal drivers in many Cornish streams – see also Gower et al., 1994), corresponding to the elimination of mayflies of these two families through the ecotoxicological effects of the reflected high metal bioavailabilities (Figure 1.13). Rainbow et al. (2012) used these threshold bioaccumulated concentrations (170 µg Cu g^{-1}, 85 µg As g^{-1}, 300 µg Zn g^{-1}, 300 µg Pb g^{-1}) to define toxic units (Adams and Rowland, 2003) for each metal by dividing the mean accumulated concentration in *H. siltalai* at each site by this threshold concentration for the relevant metal. The toxic units for different metals can be summed at each site to assess the additive ecotoxicological effects of the metals (Figure 1.14).

Schmidt et al. (2011) have addressed the same principle, and showed that whole body zinc concentrations in three aquatic insect taxa (heptageniid mayflies *Rhithrogena* spp., ephemerellid mayflies *Drunella* spp. and the caddisfly *Arctopsyche grandis*), could be used to predict ecotoxicological effects on stream communities in the Rocky Mountains, USA. Schmidt et al. (2011) were also able to define threshold bioaccumulated concentrations (critical tissue residues) of zinc in the biomonitors that were associated with specific percentage declines in mayfly densities and taxonomic completeness (the ratio of expected and observed numbers of taxa).

De Jonge et al. (2013) have extended the number of examples that show that the metal body burdens of specific biomonitors can be used to predict metal-induced effects on macroinvertebrate communities in upland streams in northwest England, using quantile regression analysis for comparison against community structure parameters including taxonomic completeness (RIVPACS) and BMWP scores. The study did not go to the preferred extent of identifying biomonitor species, but nevertheless showed that there were significant regressions between copper, zinc and lead body burdens in the stonefly *Leuctra* sp. (Zn, Pb), the heptageniid mayfly *Rhithrogena* sp. (Cu, Zn, Cu+Zn) and in mixed simuliid blackflies (Zn, Pb) and both taxonomic completeness and BMWP scores (De Jonge et al., 2013).

Using a similar approach, Bervoets et al. (2005) could relate parameters of fish community structure to summated toxic units of toxic metal accumulation in fish livers in metal contaminated river systems in Flanders, Belgium. This specific approach, however, does lack the benefit of obtaining a measure of high metal bioavailabilities in the absence of the species directly affected by those high bioavailabilities.

It appears, therefore, that bioaccumulated concentrations of metals in metal-resistant biomonitors can be calibrated to diagnose ecotoxicological impacts on stream benthos from metal stressors. There is considerable potential for further extension of the number of

biomonitors to be calibrated against observed ecotoxicological responses of benthic macroinvertebrates. In addition to the species and genera mentioned above, potential biomonitors include the zebra mussel *Dreissena polymorpha* and species of chironomid midge larvae (De Jonge et al., 2012), larvae of the caddisfly *Plectrocnemia conspersa* (Rainbow et al., 2012), mayfly larvae of the genus *Baetis*, as well as gammarid amphipods like *Gammarus pulex* in the UK and *G. fossarum* more widely in continental Europe.

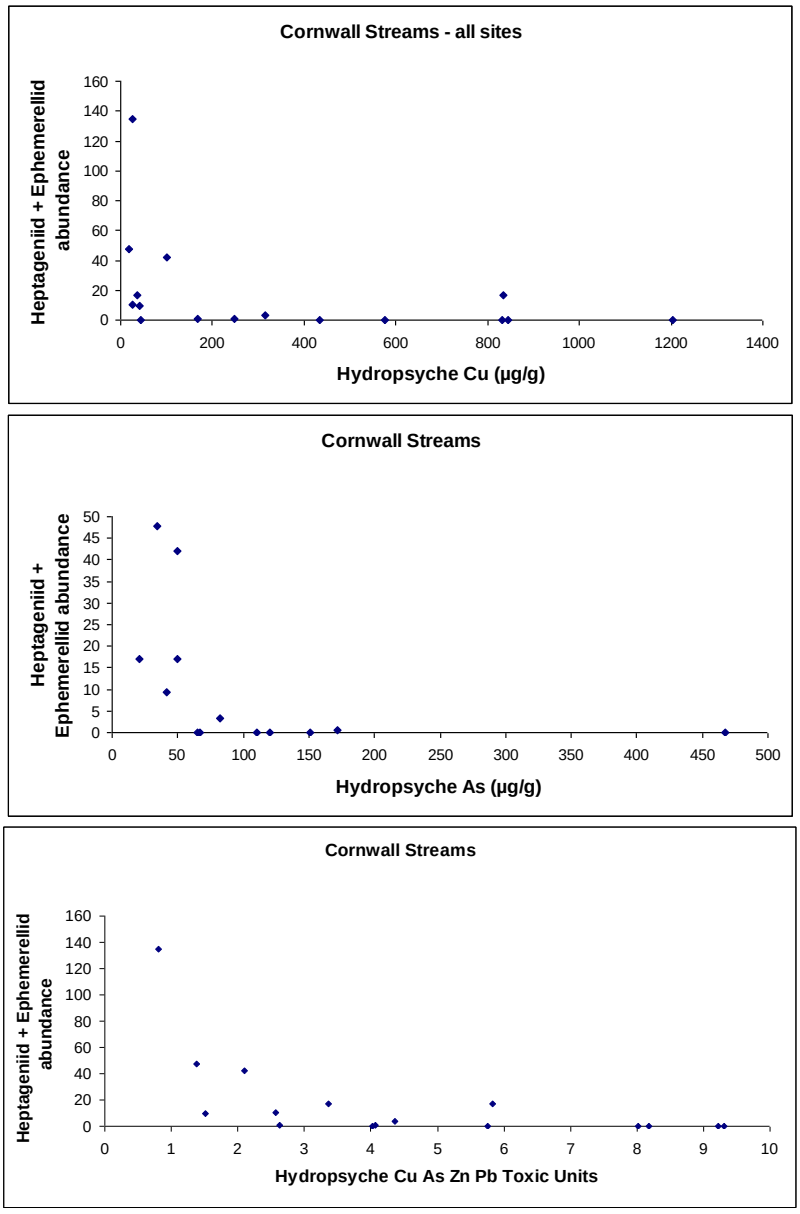


Figure 1.14. Plots of mean accumulated Cu and As concentrations, and combined mean accumulated metal concentrations expressed as toxic units, in larvae of the caddisfly *Hydropsyche siltalai* from sites in metal-contaminated Cornish rivers against combined abundance of heptageniid and ephemerellid mayfly larvae (mean number of mayfly larvae in 1 minute kick sample). (From Rainbow et al., 2012).

1.4.5 Weight of evidence (WoE) approach

The determination of the ecotoxicological potential of a metal-contaminated sediment ultimately requires the integrated use of a battery of techniques – a Weight of Evidence (WOE) assessment combining evidence from different lines of evidence (LOE) (Chapman, 2007; Benedetti et al., 2012). WOE determinations include both chemical and biological

measurements, and typically include both laboratory and field components which may be observational or involve experimental manipulation (Chapman, 2007). The original Sediment Quality Triad of Long and Chapman (1985) was a pioneer of this approach, and this triad has now been expanded, for example to include biomarkers (Chapman and Hollert, 2006; Chapman, 2007)

There are examples of the integration of laboratory and field approaches to assess the impact of metal pollution in mining-impacted streams, extending back to times before the establishment of Weight of Evidence terminology. Thus Clements and Kiffney (1994) measured water metal concentrations, bioaccumulation of metals by periphyton and selected benthic macroinvertebrates, and parameters (e.g. abundance, species richness and percentage Ephemeroptera) of the community structure of benthic invertebrates at sites in the Arkansas River, a stream impacted by historic mining activities. In the laboratory 7-day toxicity tests were carried out with the planktonic cladoceran crustacean *Ceriodaphnia dubia* exposed to water from the Arkansas River (Clements and Kiffney, 1994). In this very early integrated study, Clements and Kiffeney (1994) were able to conclude that an integrated approach be recommended for assessing effects of metals in streams. In subsequent years, Clements and colleagues took this recommendation forward with great success in the Colorado area, incorporating the more ecologically relevant results from mesocosm experiments to provide necessary toxicity test data (e.g. Scmidt et al., 2011; Clements et al., 2013).

In Europe, Wolfram et al. (2012) combined a great deal of laboratory-based and field evidence in a very impressive weight of evidence approach to assess the ecotoxicological impact of sediment contamination on benthic invertebrate communities in three river basins (Elbe, Scheldt and Llobregat). Chemical analyses of sediments were integrated with a remarkable battery of sediment toxicity tests, encompassing bacteria (*Vibrio fischeri*), benthic invertebrates of varying field relevance (the universal laboratory nematode model *Coenorhabditis elegans*, the gastropod mollusc *Potamopyrgus antipodarum*, the oligochaete *Lumbriculus variegatus*, and the midge larva *Chironomus riparius*), and fish embryos (*Danio rerio*), together with univariate and non-parametric statistical analyses of biological data on the benthic macroinvertebrates (Wolfram et al., 2012). Such biological data included the biotic indices, the Belgian Biotic Index (BBI) and the SPEAR Index (see above). A selective approach based on this study by Wolfram et al. (2012), but with the addition of biomarkers (Allan et al., 2006) has considerable potential for a WOE assessment of metal-contaminated sediments in mining-affected streams.

Recommendations for a WOE approach

It is possible finally to draw conclusions from this review of available tools for the ecotoxicological assessment of metal-contaminated sediments in mining-affected water systems and propose a WOE toolbox that would meet the requirements of the Water Framework Directive.

a Sediment Metal Concentrations

The WFD requires chemical data and it is appropriate to measure the trace metal concentrations of sediments collected from sites under investigation. These can be compared against Sediment Quality Guidelines, but it must be remembered that total metal concentrations are not measures of bioavailable metal concentrations. Extractions to model bioavailable concentrations in sediments have some value given an understanding of the routes of metal uptake used by different organisms under investigations. Indices such as the AVS index, however, are based on a flawed understanding of the biological processes affecting the uptake of metals and are to be avoided (Luoma and Rainbow, 2008; De Jonge et al., 2009).

b Bioaccumulated Metal Concentrations in Biomonitors

A real measure of bioavailable metals in a habitat is provided by the bioaccumulated metal concentrations in selected biomonitors of known biology and metal accumulation physiology and kinetics (Luoma and Rainbow, 2008). Biomonitors provide an integrated measure of the uptake and subsequent accumulation of toxic metals from different sources of metal (e.g. solution, diet) according to the biomonitor chosen, a carefully chosen suite of biomonitors covering all potential routes of uptake (i.e. bioavailable metal sources).

Biomonitors provide vital information on the geographical and temporal variations in metal bioavailabilities, in this case in a freshwater system, without direct information as to whether that biomonitor is itself suffering ecotoxicological effects of the metal exposure and subsequent metal uptake and accumulation. However, it has now been shown that absolute bioaccumulated metal concentrations in specific hardy biomonitors can be correlated with ecotoxicological changes in the habitat, and this approach, after calibration, offers enormous scope for relating the measurement of bioaccumulated concentrations in such selected biomonitors to the community structure of local benthic macroinvertebrates.

Species of the caddisfly genus *Hydropsyche* are prime candidates for this role, although other biomonitors also offer potential, perhaps in habitats where these caddisflies might be absent, for example as a result of high local sedimentation. *Hydropsyche* species spin nets to collect food, and it can be expected that fine (re)suspended sediment particles will be ingested by these caddisflies. Species of the amphipod genus *Gammarus*, such as *G. pulex* in Britain or *G. fossarum* in continental Europe, have potential as suitable trace metal biomonitors in the absence of *Hydropsyche* species at depositional stream sites (Fialkowski et al., 2003a). Other potential biomonitors are species of the stone fly genus *Leuctra* in upland streams (De Jonge et al., 2013), or species of the mayfly *Baetis* (Fialkowski et al. 2003b) which are less metal-sensitive than many other mayflies (Gower et al., 1994; Rainbow et al., 2012).

c Toxicity tests

The original Sediment Quality Triad (Long and Chapman, 1985) invoked the use of laboratory-based toxicity data, and, given their extent and availability, there is still an attraction to use such data to express comparative toxicities of metals to different organisms, not least freshwater benthic macroinvertebrates in the establishment of Species Sensitivity Distributions (SSD) or to contribute to the development of biotic indices. For assessment of sediment toxicities, laboratory sediment toxicity tests are by definition more relevant than dissolved toxicity tests. Nevertheless, even in these former tests, extra metals are often spiked to increase the range of metal exposure concentrations to be tested. Such spiking does have negative implications on the partitioning of added metals to reflect real environmental situations (Luoma and Rainbow, 2008), and a better approach might be to mix sediments from the field with high and low metal concentrations for use in toxicity tests (e.g. Casado-Martinez et al., 2010a).

More ecologically realistic and relevant data can now be obtained from *in situ* toxicity testing or mesocosm toxicity tests. These are the way forward if comparative ecotoxicity data are required in a particular WOE assessment.

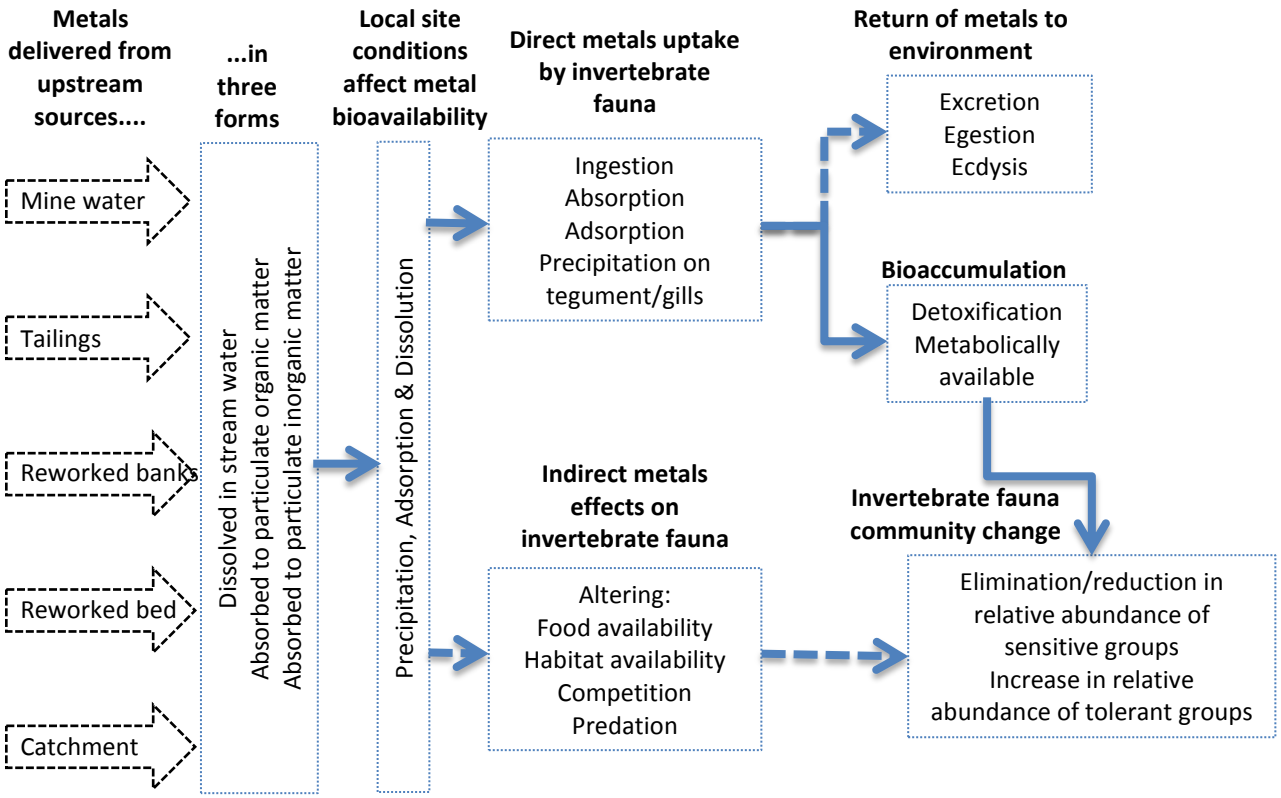


Figure 1.15. Schematic diagram of how metals derived from mine workings and the catchment impact the invertebrate community. The bioavailability of metals from upstream sources is influenced by local site conditions, which influences the direct uptake of metals by invertebrate fauna (primarily through ingestion of particulates). Some of the metals taken up are returned to the environment, the remainder are bioaccumulated (either detoxified in the tissues or metabolically active and, hence, toxic). The toxicity of available metals results in change in the invertebrate fauna. Although toxicity of metals taken up by direct consumption is the main route of impact, indirect effects of metals can also impact the invertebrate fauna. For simplicity feedback loops are not illustrated (e.g. bioaccumulation of metals alters food availability and competition [by elimination/reduction of sensitive groups], and the metal content of available prey, which combine to alter the rate of metal uptake through ingestion).

d Biomarkers

Biomarkers do provide evidence of the presence of an ecotoxicological effect on metal-exposed organisms, and have great potential for successful inclusion in biological monitoring programmes (Allan et al., 2006; Chapman and Hollert, 2006; Collier et al., 2013; Amiard-Triquet et al., 2015).

Biomarkers at the lower levels of biological organisation (molecular, biochemical and cytological) are more sensitive than the more obviously ecologically relevant biomarkers at higher levels (e.g. population), have been correlated with higher organisational level effects (Moore et al., 2013) and are not difficult to measure in these days of 'omics'. Without resource to 'omics', attractive biochemical-level biomarkers include primary antioxidant enzymes like catalase, the oxyradical scavenger glutathione, and the Total Oxyradical

Scavenging Capacity (TOSC). The metal-binding protein biomarker should only be employed with care given the variability in its induction properties. Of the cytological biomarkers available, the measurement of lysosomal stability using the neutral red retention (NRR) assay has much to recommend it (Moore et al., 2013), after extension to suitable candidates from relevant stream biota. Invertebrates with blood cells are the best candidates, and so might include *Gammarus* species, chironomid midge larvae or tubificid oligochaetes. In the latter case it would be possible to draw on the extensive experience of soil ecotoxicologists using the NRR assay in earthworms, the oligochaete relatives of freshwater tubificids (Spurgeon et al. 2003; Svendsen et al., 2004). Biomarkers of genotoxicity that are well tested include the comet assay.

Moving up the hierarchy of biological organisation, potential metal-affected biomarkers at the level of the organism are the distorted morphology of benthic diatoms (Falasco et al.; 2009) and the morphology of the mouthparts of chironomid midge larvae (Martinez et al., 2003; Di Veroli et al., 2014)

e Biotic index

Biotic indices are widely used to meet WFD requirements for assessment of biological quality elements in freshwaters, and historically have long been used with benthic macroinvertebrate communities to assess the extent of pollution. Such biotic indices, however, have typically been developed to address organic pollution, although general degradation may be interpreted more generally (e.g. through NTAXA). A European biotic index for metal pollution is still to be developed (SNIFFER, 2011a), a non-trivial task. However the Australian experience with a diagnostic index for acid mine drainage, SIGNAL-MET (Chessman and McEvoy, 1998), in combination with a RIVPACS style predictive model of community structure, where 70% of the variation in observed/expected was attributable to mining impacts, indicates that this approach is highly appropriate for assessing the degree of impairment from previous mining activity (Sloane and Norris, 2003).

In the absence of a specific biotic index for metal-contaminated sediments, several authors have used a comparative multimetric statistical approach for the comparison of macroinvertebrate communities at stream sites under different conditions of metal exposure (e.g. Gower et al., 1994; Hirst et al., 2002; De Jonge et al., 2008). Archaimbault et al. (2012) have used a multimetric approach to assess the ecotoxicology of contaminated sediments to benthic macroinvertebrates in French mountain streams, but this study considered a mixture of toxicants, involving organic contaminants such as PAHs and PCBs as well as toxic metals.

A biotic index developed by linking community level responses to measures of bioavailability and ecotoxicological responses (Figure 1.16) would provide the best route for a rapid, easy to use, and WFD compliant method to assess the impact of metal-contaminated sediments on biota.

1.4.6 Remediation

Together with the need to identify where sites are being impacted by metal-contaminated sediments, there is a clear ongoing need for a low cost system that is capable of assessing the effectiveness of any remediation undertaken to mitigate the impact of metal-contaminated sediments. This system should be capable of rapid and easily repeatable assessments that can be undertaken through time to determine the rate of improvement of mining-affected stream sites after mitigation, and in particular after any event that may influence the delivery of metal-contaminated sediment from the catchment.

1.5 Conclusions

It is clear that the way forward in the ecotoxicological assessment of mining-affected stream sediments is via an integrated approach involving a multiplicity of tools from different disciplines such as biology, ecology, geochemistry and toxicology (Luoma and Rainbow, 2010), as reflected in a Weight of Evidence approach (Chapman, 2007).

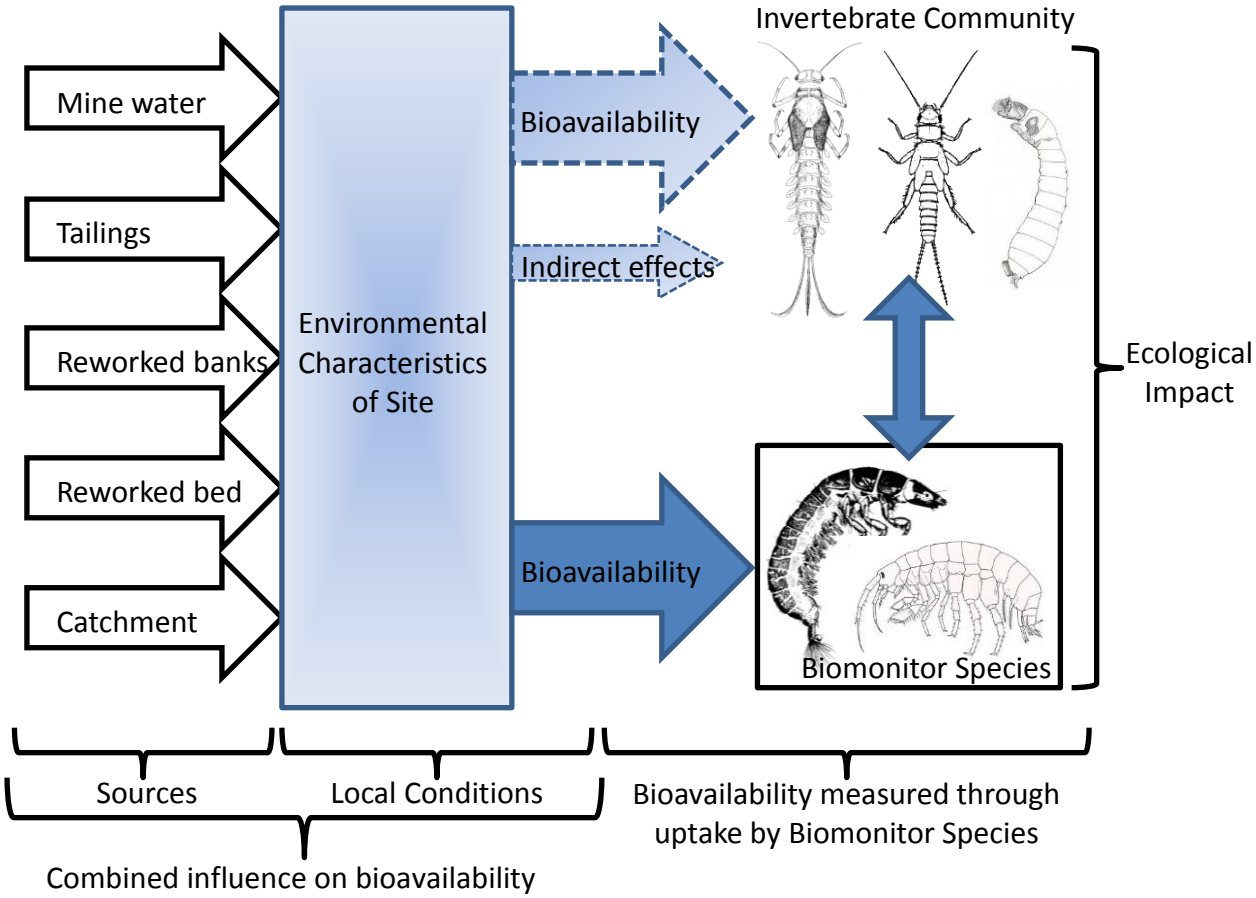


Figure 1.16. Model of approach proposed here to assess the ecological impact of metals. The metal load at a site is derived from various sources, both current and historic, and the bioavailability of this load is affected by local environmental conditions. The invertebrate community comprises many species, some of which will be removed by metal pollution. Hence, the bioaccumulated concentrations of metals in tolerant biomonitor species can be used as a measure of bioavailability, and this measure of bioavailability used to diagnose ecological impacts from metal stressors on the whole stream benthos community.

2 Compilation of Existing Data

Objective 1b

To compile existing water chemistry, sediment chemistry and biological data from potentially impacted stream and river sites (Environment Agency, 2008b,c; British Geological Survey Geochemical Baseline Survey of the Environment (G-BASE)).

2.1 Sources of data compiled

Data have been compiled from the following sources:

BGS G-BASE	– sediment metal chemistry
	– water metal chemistry
BGS G-BASE SW Tellus	– sediment metal chemistry
EA/NRW biological monitoring data	– invertebrates
	– diatoms
	– macrophytes
	– fish
EA/NRW chemical monitoring data	– sediment metal chemistry

2.1.1 G-BASE

The British Geological Survey's Geochemical Baselines Survey of the Environment (G-BASE) project is a systematic high resolution survey to establish a geochemical baseline across the United Kingdom. The regional geochemical sampling programme began in 1968 in the northern Highlands of Scotland and has progressed southwards (Figure 2.1), with the aim of producing maps to show the distribution of trace elements in soils and stream sediments. As spatial coverage is a priority in this project, low-order streams are targeted.

Stream sediment was collected with a trenching tool and, excluding the uppermost heavily oxidised sediment, was wet sieved at site to <150 µm (Johnson, 2005). A duplicate field sample was collected from one site in 100 for quality control purposes. Sediments are dried initially by air drying then freeze drying before being pulverised in agate ball mills. Samples are pelletised ready for X Ray Fluorescence Spectroscopy (XRFS) at the BGS laboratories in Keyworth, UK. During the lifetime of the project analytical, statistical and data processing techniques have evolved substantially. Initial stream sediments were analysed for 16 elements using Direct-reading DC Arc Optical Emission Spectrometry (DCOES), Atomic Absorption Spectroscopy (AAS) and (for U) delayed neutron activation. In the Hebrides and subsequent atlas areas (including Northern England) a direct-reading emission spectrometer was used to determine some 25 elements. The current analytical method is XRFS, which commenced on the Welsh sediments and determines 48 elements, namely Ag, Al, As, Ba, Bi, Br, Ca, Cd, Ce, Co, Cr, Cs, Cu, Fe, Ga, Ge, Hf, I, K, La, Mg, Mn, Mo, Na, Nb, Nd, Ni, P, Pb, Rb, Sb, Sc, Se, Si, Sm, Sn, Sr, Ta, Te, Th, Ti, Tl, U, V, W, Y, Zn and Zr.

A total of four spot samples of filtered (0.45 µm cellulose filter) and unfiltered surface water are collected from each site at the same time as the stream sediment. Samples are stored in Nalgene™ bottles and acidified as required by the analytical method. Alkalinity (by colorimetric titration), pH and conductivity determined on site. Samples are analysed at BGS

and variously include Inductively Coupled Plasma Mass Spectrometry (ICP-MS) for Ag, Al, As, Ba, Be, Bi, Cd, Ce, Co, Cr, Cs, Cu, Ho, La, Li, Mo, Mn, Nd, Ni, Pb, Rb, Sb, Se, Sn, Th, Tl, U, V, Y, Zn & Zr [31 elements]; Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) for Al, B, Ba, Ca, Fe, K, Mg, Mn, Na, P, Si, S (reported as SO_4), Sr & Zn [14 elements]; Ion Chromatography for Br, Cl, F, NO_2, NO_3, SO_4, PO_4 [7 ions] and a TIC/TOC analyser for Non-purgeable organic carbon (NPOC). Again, the range of determinands analysed has varied as methods have evolved over the life of the project.

Data were requested from BGS describing the site location, sampling date, stream sediment geochemistry and stream water chemistry. Data from 61,195 locations sampled 1985 – 2008 were provided (Table 2.1). Due to evolution of techniques, the analysis used and elements reported vary among sites: only those sites where the suite of determinands analysed included metals were retained. The data were housed in an MS Access data base.

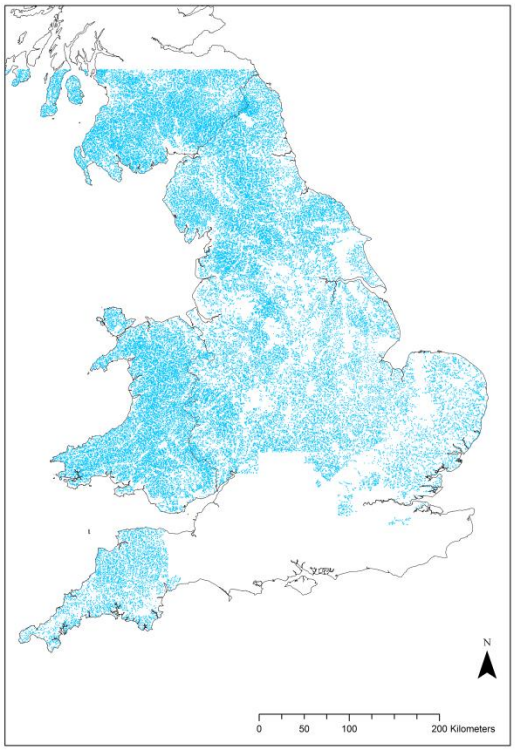


Figure 2.1. Map of G-BASE stream sample locations in England and Wales including the data from the SW Tellus project.

Following the release of data from the SW Tellus project covering Devon and Cornwall, a further data request was made to BGS describing the site location, sampling date and stream sediment geochemistry. Data from 3,779 sites sampled 2002 – 2012 were provided (Table 2.1). The data were housed in the MS Access data base.

2.1.2 EA and NRW – biological monitoring data

Data describing the Biological Quality Elements (BQE) invertebrates, diatoms, macrophytes and fish have been collected using the appropriate field sampling methods by the regulatory authorities to assess the biological condition of rivers; although in some cases the samples

predate the adoption of the WFD, the field methods have remained largely unchanged. As the aim of this monitoring is to report biological condition of rivers the samples have largely been collected from main channels of river catchments (Figure 2.2); this concentration on main stem sites is particularly pronounced for fish. These data are held by the EA/NRW in the BIOSYS (invertebrates, diatoms, macrophytes) and fish databases.

The data on community composition have been used to derive biotic indices to summarise the quality of the site, namely

Invertebrates	NTAXA	= number of BMWP (Biological Monitoring Working Party) families present
	ASPT	= Average BMWP Score Per Taxon present
Diatoms	TDI	= Trophic Diatom Index
	Number of species [not used for WFD classification]	
Macrophytes	RMNI	= River Macrophyte Nutrient Index
	Number of species [not used for WFD classification]	
	Number of aquatic species	
Fish	Number of species	

Since the adoption of the WFD the data have been used to calculate the Ecological Quality Ratio (EQR) for the site by comparing returned values for the site to those values expected if the site were in reference condition.

All available routine biological monitoring data up to 2012, comprising macroinvertebrate, macrophyte, fish and diatom community composition, and derived biotic indices, together with site details and environmental characteristics were requested from the EA/NRW. EQR values were requested for sites/sampling occasions where these data were available. The number of sites and number of samples collected varied dependent upon BQE, with diatoms covering the least number of sites and samples (Table 2.1). The time periods covered for the different Biological Quality Elements (BQE) also vary: fish 1975-2012, macroinvertebrates and macrophytes1994-2012, diatoms 1998-2012. The data were housed in an MS access database (see Figure 2.3 for example of structure).

Where indices have been introduced since the adoption of the WFD [e.g. RMNI has replaced the Mean Trophic Rank index], on receipt of the data WFD compliant index values were derived using species composition data for samples collected prior to changes. Where taxonomic resolution has changed over time, index values appropriate for the resolution used at the time were used.

2.1.3 EA and NRW – chemical monitoring data

In order to assess the condition of rivers, samples of water and sediment have been collected by the regulatory authorities. These samples have been used to determine various hydrochemical parameters, including metal content of water and sediment, with samples variously collected over multiple occasions from each location in order to establish exceedance (Table 2.1). Both water and sediment hydrochemical data are stored in the same database. However, sediment has been collected from a small number of sites compared with water chemistry: sediment data are related to specific issues rather than

collected systematically as part of an established national monitoring programme. Results of sediment metal concentrations have been reported variously for different particle size fractions. Where information is available it appears that metal content has been established largely on the fine size fraction (e.g. <63 µm, <90 µm) after acid extraction.

All available river chemistry monitoring data, including trace metals concentrations in sediments, were requested from the EA/NRW, together with site location and sampling date. Data were housed in MS Access databases. Nine separate databases were used each covering a single EA/NRW region (with 2 for the Midlands) and linked together by a hub. Those data describing trace metals concentrations in sediments were extracted from these holdings.

Table 2.1 Summary of datasets requested

Organisation	Dataset	Earliest sampling date	Latest sampling date	No of sites	No of samples/surveys
BGS	G-BASE sediment	24/06/1985	11/07/2008	61,195	61,195
	G-BASE water	26/06/1986	09/08/2007	24,206	24,206
EA/NRW	Invertebrates	03/01/1994	11/12/2012	15,155	121,243
	Diatoms	21/08/1998	30/11/2012	2,837	6,582
	Macrophytes	05/08/1994	10/10/2012	4,592	8,979
	Fish	19/05/1975	13/12/2012	15,268	48,077
EA/NRW	Water Chemistry	01/01/1990	12/12/2012	27,050	2,725,344
	Sediment Chemistry	01/01/1990	12/12/2012	964	9,053
BGS/Tellus	G-BASE SW sediment	2002	2012	3,779	3,779

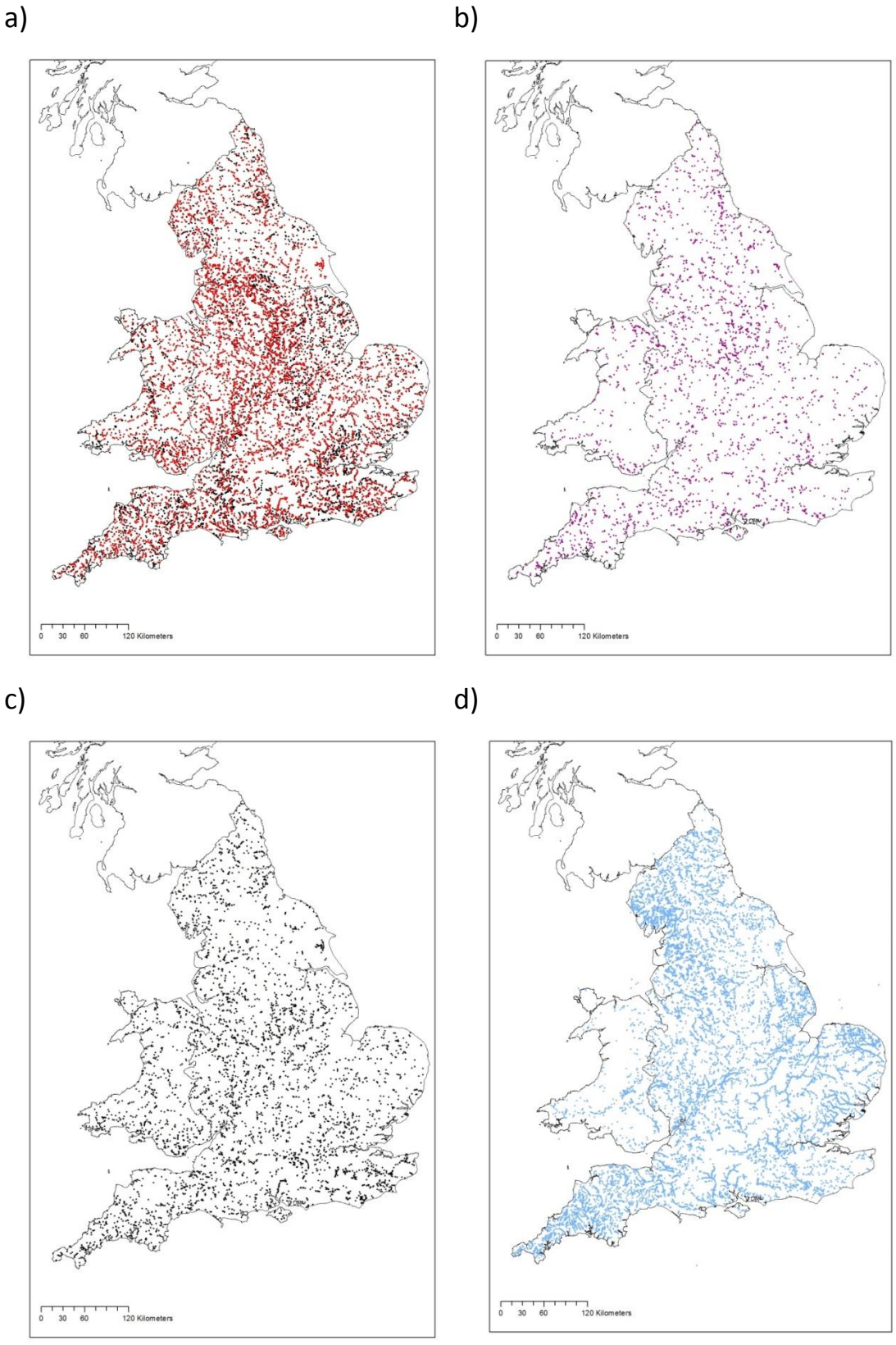


Figure 2.2. Map of EA/NRW river biological sample locations in England and Wales a) macroinvertebrates, b) diatoms, c) macrophytes, and d) fish.

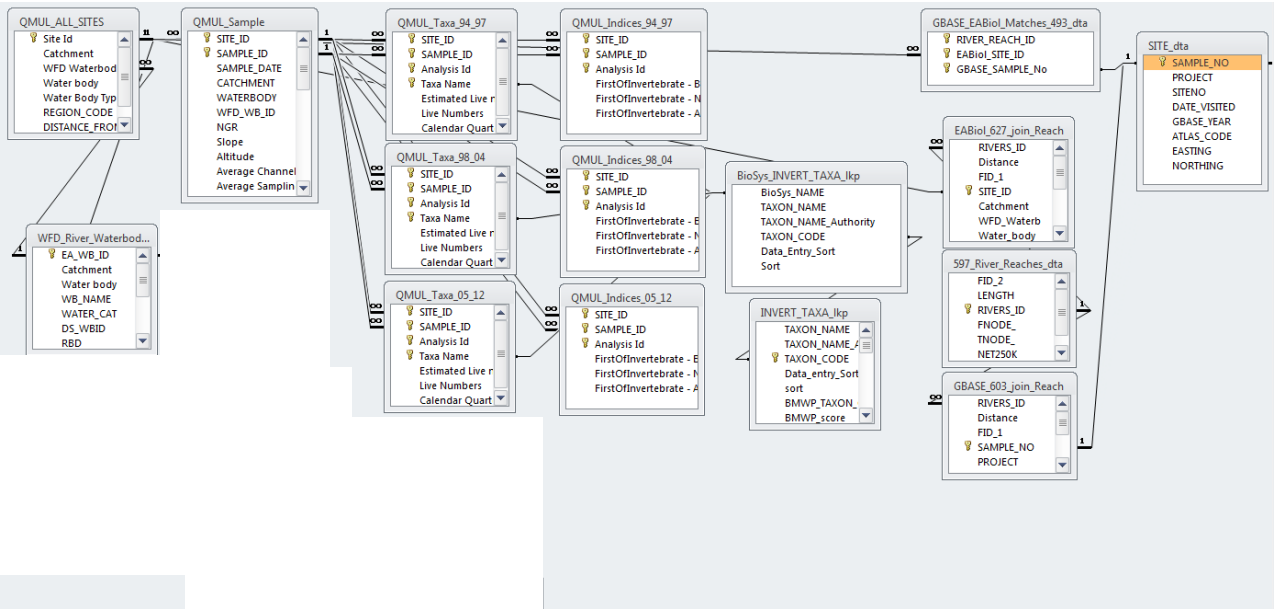


Figure 2.3. Structure of database housing EA/NRW invertebrate data matched to G-BASE data.

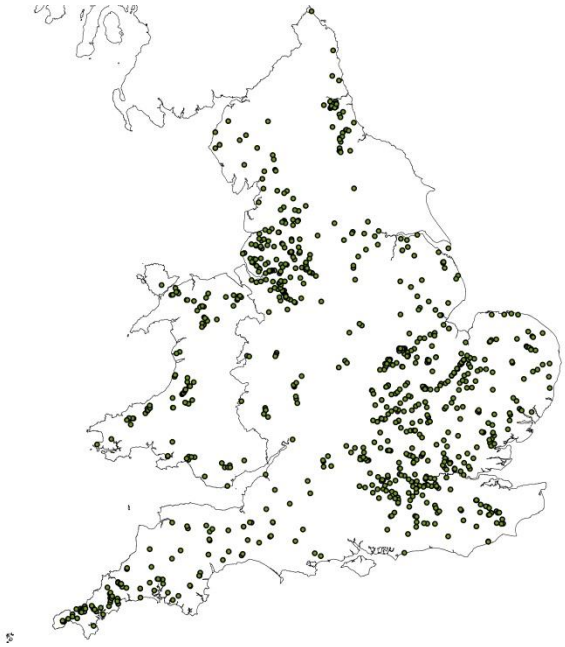


Figure 2.4. Map of EA/NRW river sediment sample locations in England and Wales.

2.2 Spatial matching

Biological data were matched spatially to data describing sediment chemistry in ArcMap 10.2 using the river network. Sample site locations were plotted together with the river network based on the OS 1:25,000 map. The “blue line” of the river network was divided into segments where each segment comprised a continuous section of a single river channel without any joining channels: divisions between segments occurred at the confluence of channels (tributaries, anastomosing channels). Hence, each segment represents a continuous river channel without inflows (as indicated on the OS 1:25,000 map). Biology and chemistry sites were matched if they both intersected with the same river segment (Figure 2.4). A buffer of 50 m was added to the river network, i.e. sites were regarded as intersecting with a segment if they occurred within 50 m of that segment of the blue line. This allowed for imperfect location of sites and for the fact that the “blue line” may not accurately represent the full width of the channel. However, sites could be erroneously clipped to a river segment if they were located near a confluence. Manual checks were undertaken to avoid such errors. Segments that contained multiple biological or chemical sampling sites were also checked manually, and where multiple sites were verified as matching with the same river segment the pair of sites representing the closest spatial match was chosen. Site identities were then used to match chemical and biological data using MS Access.

To ensure that sites with high sediment metal concentrations were included wherever possible a further directed search was made. G-BASE sites were ranked according to their sediment cadmium, copper, nickel, lead and zinc concentrations and the 1483 sites with exceptionally high levels (> Probable Effect Level: Canadian Council of Ministers of the Environment, 1999) for one or more of these elements were selected. These G-BASE sites were linked to river segments (as above) and the closest EA/NRW biological sampling sites to these river segments identified and checked manually. Most biological sampling sites were too far from the river segment to provide a valid match to the G-BASE sites and a further sub-set had already been identified and matched. However, a small number of additional sites (47 for invertebrates) were identified that had been missed in the original screening and the spatial matching was sufficient to warrant inclusion.

Once sites had been matched spatially the BQE sampling year which represented the best temporal match with the chemical sampling occasion was chosen. Where multiple BQE samples were collected within the year that represented the best temporal match, the sample with the highest index value was chosen as representative of the condition of the site.

Despite the high density of G-BASE sample sites there was, to some extent, a spatial mismatch between the G-BASE and EA/NRW biological monitoring sites, with the former focussing on headwaters and the latter on the main stem. Nevertheless, for invertebrates and fish, matches were found for a substantial number of sites (Table 2.2).

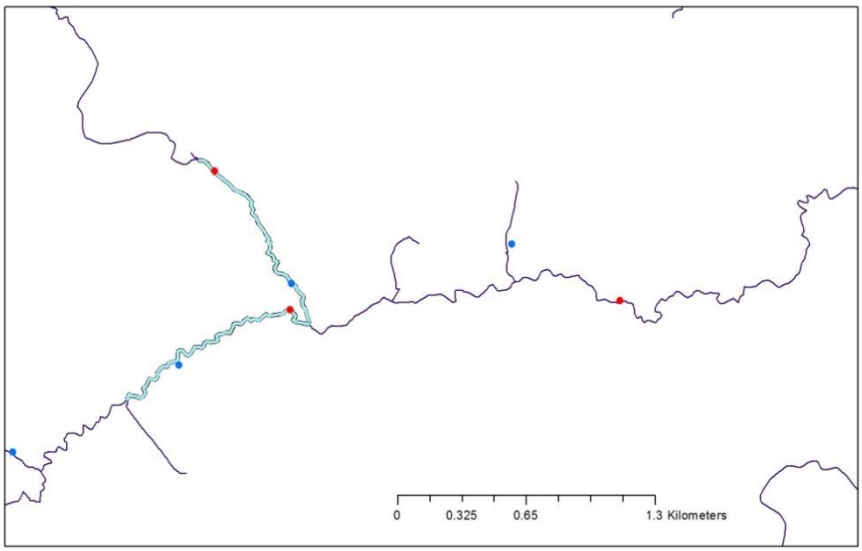


Figure 2.4. Example of spatial matching of G-BASE sites (blue dots) to EA/NRW biological sampling sites (red dots) using the river network (blue line). The two segments containing matched sediment chemistry – biology sites are marked in bold. Note that the two sites near the confluence of the segments containing matched sites have been correctly paired within their segments, despite these sites being spatially close to one another.

Table 2.2 Number of sites with matched biology and sediment chemistry data.

	G-BASE				EA/NRW	
	Sediment chemistry*		Water chemistry*		Sediment chemistry°	
	Sites	Samples	Sites	Samples	Sites	Samples
Invertebrates EQR	1,377 963	1,776 963	995 589	1,258 589	178 97	902 152
Diatoms EQR	324 189	539 189	278 151	453 151		
Macrophytes EQR	439 209	439 209	372 161	376 161		
Fish EQR	1,367 1,367	1,367 1,367	1,184 1,184	1,184 1,184		

* numbers shown are for all sites/samples, actual numbers vary dependent upon element.
° numbers shown are for cadmium, number of matched sites/samples are considerably lower for other elements.

Due to the evolution of methods during the lifetime of the G-BASE project it was necessary to level the concentration of metals and metalloids in stream sediments determined using XRFS and DCOES so that concentrations were not influenced by analytical method.

A similar process of matching EA/NRW sediment chemistry and biology sites using river segments was undertaken. It was decided to keep these data separate from the matched biology – G-BASE sediment chemistry data due to differences in the analytical approaches used and size fractions analysed between EA/NRW and BGS. Considerably fewer EA/NRW sediment chemistry sites provided matches to the biology than did G-BASE (Table 2.2), with the number of matches varying across the different metals. The most abundant EA/NRW biology – sediment chemistry matched data was for cadmium and the least for arsenic.

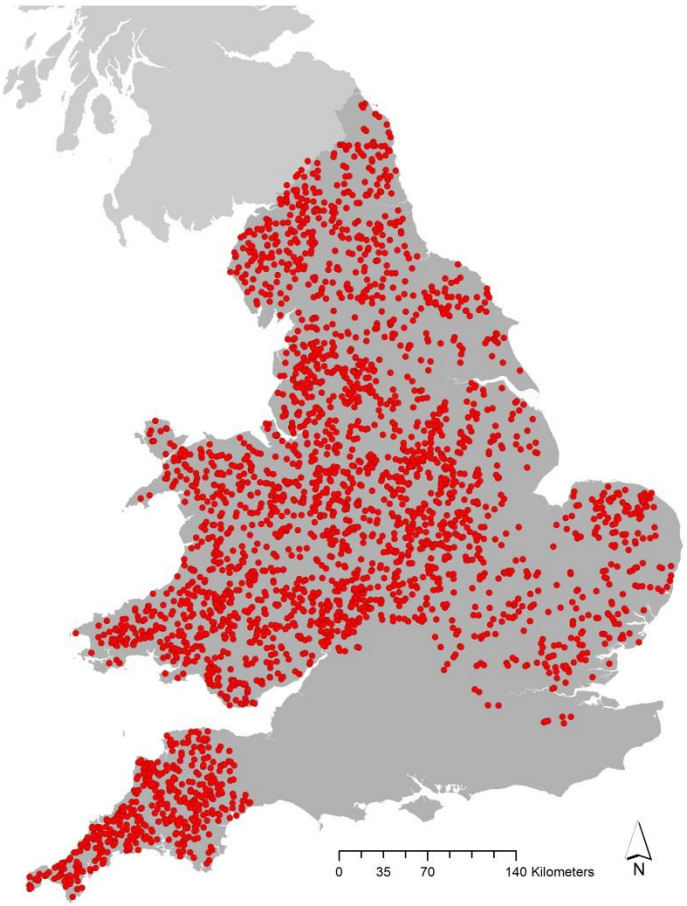


Figure 2.5. Map of all sites with matched G-BASE sediment chemistry and EA/NRW biological data (total number of sites = 2833).

2.3 Analysis of G-BASE data

As the purpose of compiling the existing data was to produce a matched sediment chemistry – biology dataset for further analysis, it was necessary to develop our understanding of the variation in sediment metal concentrations in the G-BASE dataset. The initial analysis involved a pairwise comparison of variation in the elements of interest, namely the metals silver, cadmium, chromium, copper, nickel, mercury, lead, tin and zinc, and the metalloids arsenic and antimony. Here all the G-BASE sediment chemistry data available were used as the objective was to establish the co-occurrence of elements and, hence, identify any potential confounding effects that should be considered when interpreting any relationships based on these data.

Several elements were correlated in the G-BASE data (Figure 2.5). Of particular note were arsenic, cadmium, copper, nickel, lead and zinc which tended to co-occur. Nevertheless, there did appear to be sufficient variation within the relationships between elements to enable the influence of each element to be assessed independently. There were considerably fewer data describing the occurrence of mercury in stream sediments than other metals or metalloids, making it difficult to establish any relationships between mercury and other elements (Figure 2.5).

To understand better the range and distribution of concentrations of metals and metalloids in stream sediments, a frequency distribution plot was derived for each element (Figure 2.6a-c). Here the data describing sediment concentrations of each element were ranked and the percentile position of each sample within this ranking calculated.

To enable the stream sediment metal concentrations to be put into context, the frequency distribution of sites (samples) were compared with the Canadian interim sediment quality guidelines (Canadian Council of Ministers of the Environment, 1999) and the Australian and New Zealand Low Trigger Value (ANZECC and ARMCANZ, 2000) where available. Thus, the proportion of sites (samples) exceeding these sediment quality guidelines can be established. However, it should be noted that for certain elements the geochemical analytical methods used by BGS (XRFS/DCOES/AAS) may return higher metal concentrations than analyses based on acid extraction (typically undertaken for toxicological analysis), as the geogenic component derived from the underlying parent material (geology) may be included in the former.

Again it was clear that the low frequency of data describing mercury in stream sediments within G-BASE limits the conclusions that can be drawn for this element (Figure 2.6b). Whilst there were fewer sites with data describing antimony, cadmium and silver concentrations than for the other elements, which resulted in rather abrupt changes in the frequency distribution for these elements, there was sufficient to draw reasonable conclusions.

With the exception of mercury, the Canadian interim sediment quality guidelines are more prescriptive than the corresponding Australia and New Zealand low trigger values. This is reflected in the proportion of sites where sediment metal/metalloid concentrations measured by G-BASE were in exceedance of these limits, with more sites in exceedance of the Canadian guidelines (Table 2.3). For chromium, nickel and possibly arsenic, the exceptionally high proportion of sites exceeding the guideline concentrations suggests that the G-BASE methods may not be compatible with those used to derive these standards for

these elements, more reflecting the mineralogy than the biologically available component. Nevertheless, it is apparent that for most metals a relatively large proportion of sites sampled in G-BASE are in exceedance of these guidelines, suggesting that the data should be adequate to detect effects on biota (Objective 1b).

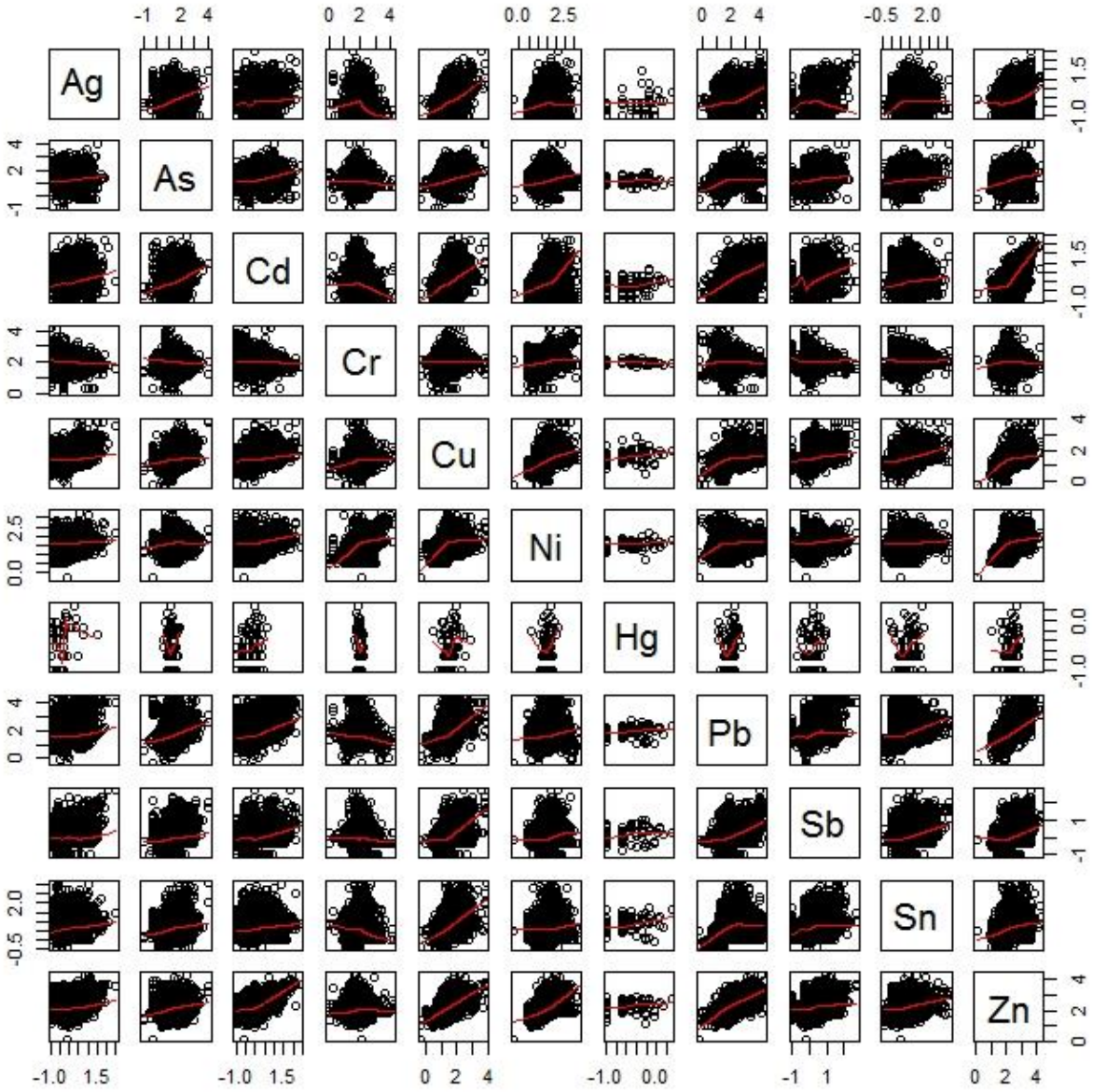


Figure 2.5. Pairwise correlations showing co-occurrence of metals and metalloids in stream sediments, from G-BASE.

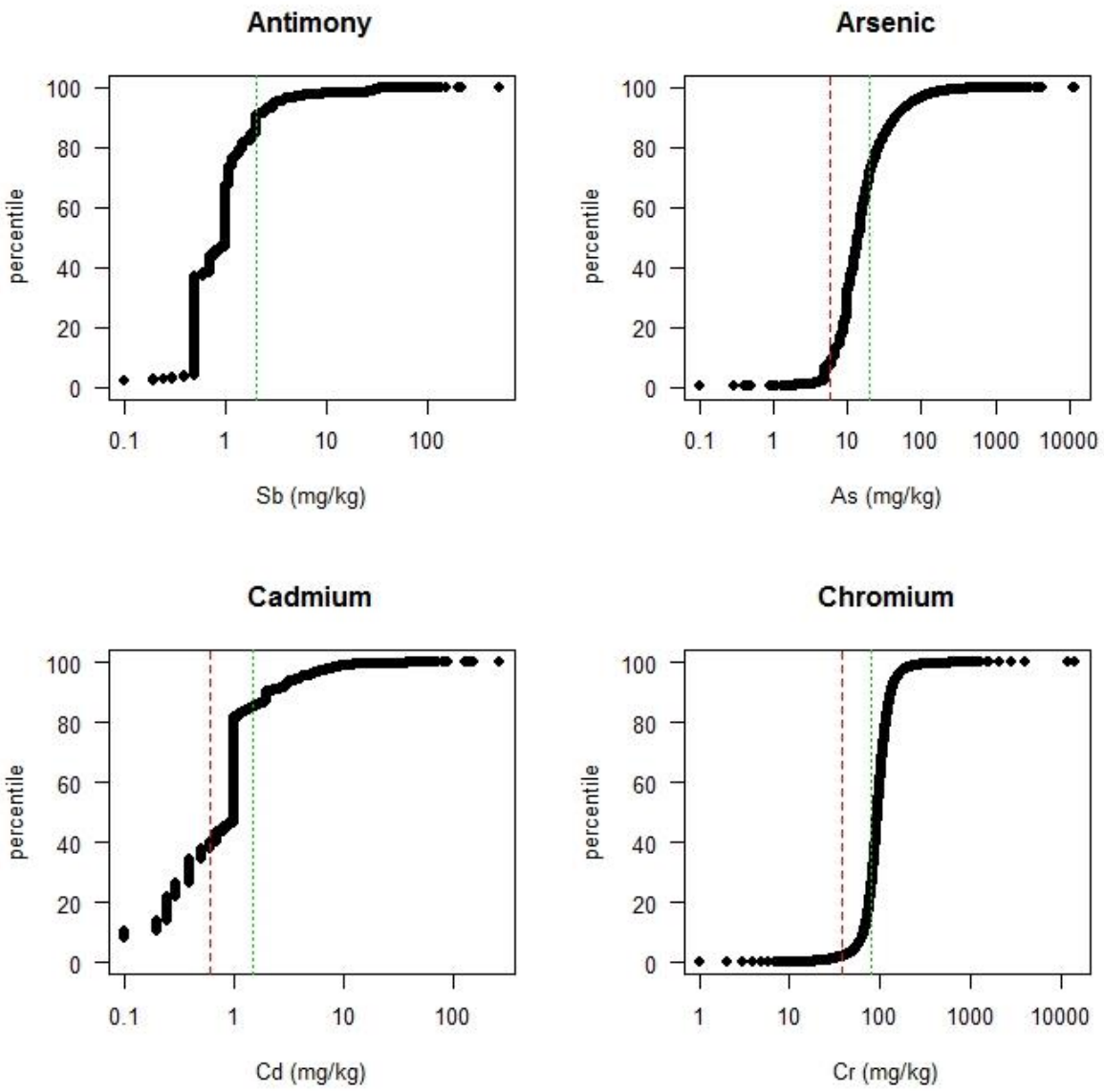


Figure 2.6a. Frequency distribution plots of antimony, arsenic, cadmium and chromium in stream sediments (from G-BASE). Also shown are the concentrations of the Canadian interim sediment quality guidelines (red dashed line) and the Australian and New Zealand low trigger value (green dotted line) where available

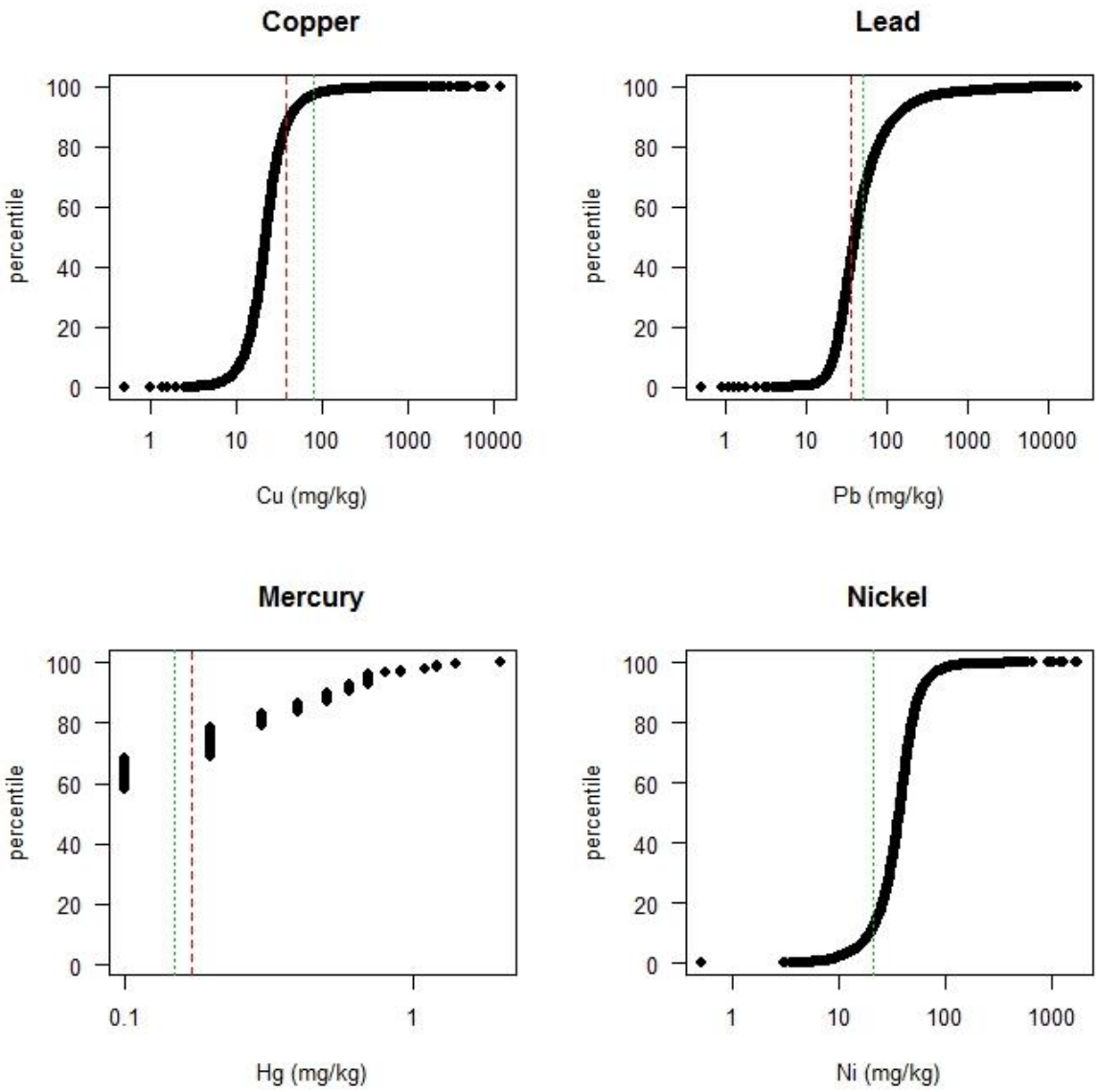


Figure 2.6b. Frequency distribution plots of copper, lead, mercury, and nickel in stream sediments (from G-BASE). Also shown are the concentrations of the Canadian interim sediment quality guidelines (red dashed line) and the Australian and New Zealand low trigger value (green dotted line) where available.

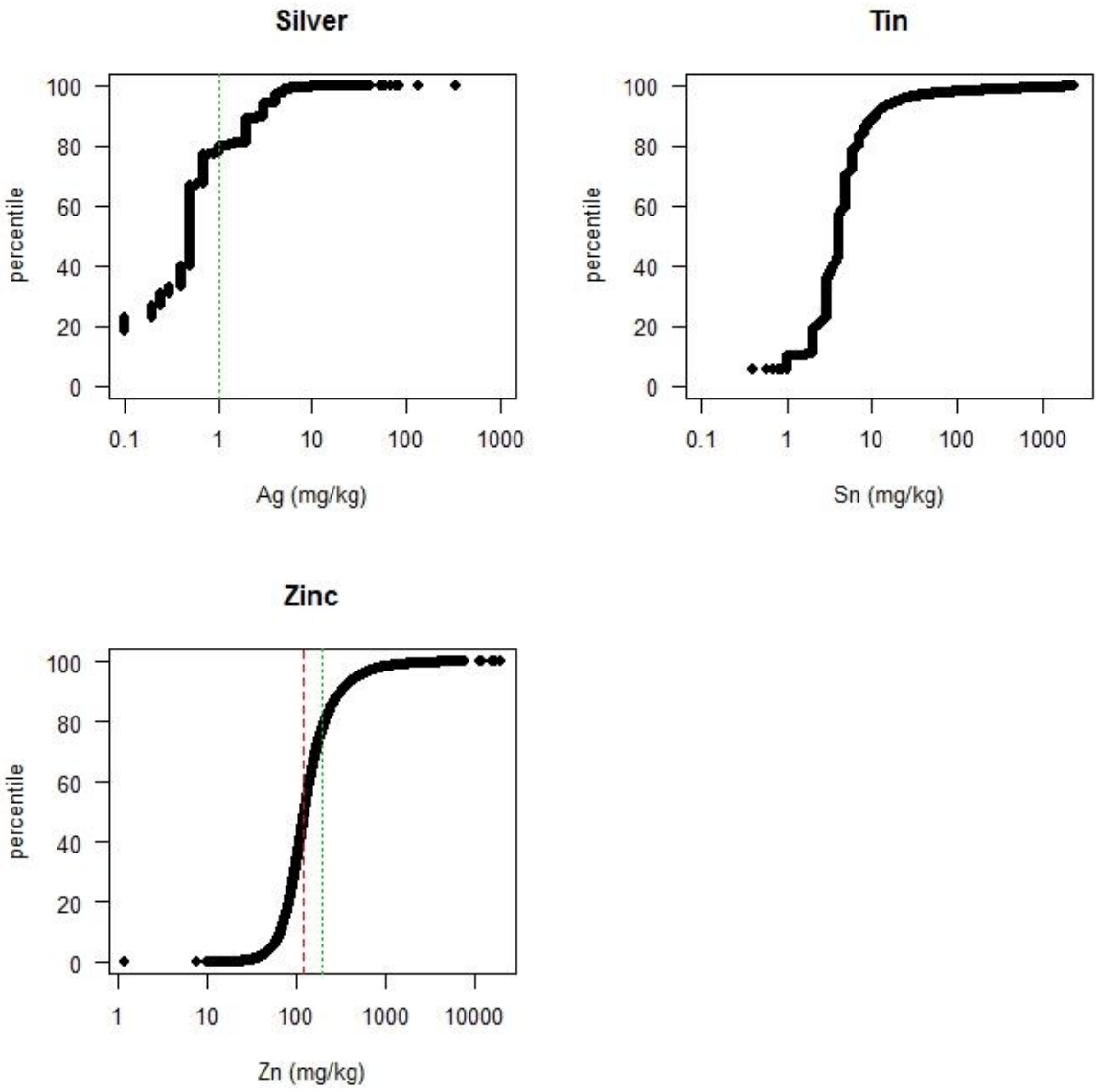


Figure 2.6c. Frequency distribution plots of silver, tin and zinc in stream sediments (from G-BASE). Also shown are the concentrations of the Canadian interim sediment quality guidelines (red dashed line) and the Australian and New Zealand low trigger value (green dotted line) where available.

Table 2.3. Percentage of G-BASE sites where sediment metal concentrations were in exceedance of the Canadian interim sediment quality guidelines and the Australia and New Zealand low trigger values.

		Percentage of G-BASE sites in exceedance		
		Australia and New Zealand: Low trigger value	Flanders: Sediment quality guidelines	Canada: Interim sediment quality guidelines
Antimony	Sb	9.2	–	–
Arsenic	As	28.7	32.2	92.4
Cadmium	Cd	15.1	18.0	59.9
Chromium	Cr	75.8	–	98.1
Copper	Cu	4.3	59.4	16.2
Lead	Pb	37.3	50.5	60.0
Mercury	Hg	31.3	9.5	31.3
Nickel	Ni	88.0	93.9	–
Silver	Ag	20.2	–	–
Tin	Sn	–	–	–
Zinc	Zn	22.4	38.6	52.4

[– guideline concentrations not available]

3 Analysis of Existing Data

Objective 2a

To analyse existing ecological (macroinvertebrates, benthic algae, macrophytes and fish) and chemical (water and sediment) monitoring data from water bodies at risk to sediment-borne mining-related metal contamination (Environment Agency, 2008b) to identify if and where such stressors result in sites failing to achieve EU WFD good ecological status.

3.1 Methods

The spatially and temporally matched sediment chemistry – biology datasets compiled in objective 1b were used to explore the relationships between sediment chemistry and biological response. As the toxic effects of trace metals were not expected to occur until the uptake rate has exceeded the combined rates of efflux and detoxification (Luoma and Rainbow, 2008) a threshold biological response to sediment metal concentration was expected (see Figure 1.4). It was also assumed that where sediment metal concentrations were below the threshold, other factors could potentially influence the biological response. Hence, quantile regression was used where the 95%ile was modelled using a threshold response of biological variables where there was no influence of sediment metal content below a cut-point concentration (Figure 3.1). Relationships were modelled in R version 3.2.0 using the pricefit procedure, an iterative parameter optimisation procedure using the pseudo-random search algorithm of Price (1997). R code for the modelling procedure is given in appendix 2. Three parameter values were obtained using this procedure (Figure 3.1).

a – The 95%ile below the threshold. A fixed value of the biological response variable describing the upper range of values obtained at sites where the sediment metal concentration does not cause toxic effects.
b – The threshold sediment metal concentration. A fixed value of the sediment metal concentration above which the sediment metal concentration constrains the upper range of biological response values, i.e. the threshold above which toxic effects are apparent.
c – The slope of the 95%ile of the biological response above the threshold. At these sediment metal concentrations toxic effects are increasingly apparent. The slope of the relationship above the threshold is determined by how toxic the metal is.

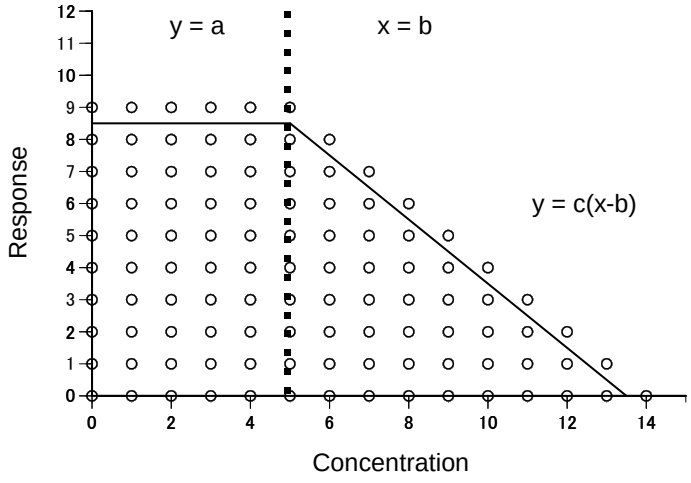


Figure 3.1 Schematic diagram illustrating the threshold relationship expected between sediment metal concentration and biological response variables and the three parameters fitted using quantile regression and the pricefit procedure.

The significance of the threshold model was determined using Akaike's Information Criterion (AIC) values calculated based on the sum of weighted absolute deviation using the method described in Pacheco et al. (2005). AIC values for the threshold model were compared with values obtained for an exponential, a linear and a null model. The threshold model was selected as optimal if it had the lowest AIC values and differences in AIC values from other models were >2 (Anderson and Burnham, 2002). It was noted where AIC values indicated that the threshold model was not optimal as defined by these criteria, but was not worse than optimal model, i.e. where the difference in AIC values was between 0 and 2. Also, it was noted where the threshold model was selected as optimal but the parameter estimates did not fit with the assumptions of toxic effects (Figure 3.1), i.e. parameter c was positive.

Of the three modelled parameters, the value of b – the threshold sediment metal concentration – is of most interest. This value represents the concentration above which a negative relationship is apparent between sediment metal concentration and biological response values, suggesting that the toxic effect of the metal are being expressed.

Biological response data were summarised as indices used for WFD classification, other indices that are used to interpret WFD classifications (although not used in the classification *per se*), taxonomic richness (where this is not used as an index for WFD classification), and as Ecological Quality Ratios (EQR) which are the value of the WFD index compared with the expected value of the index if the site were in reference condition (i.e. observed/expected). As EQR values were obtained from the EA/NRW data holdings, values were only available for those data collected after the adoption of the WFD. As models were fitted to the 95%ile, where multiple biological samples were collected in the year that provided the best temporal match to the chemical sampling occasion, the highest value of the biological response was used (following from the expectations of the response modelled – see Figure 3.1).

Matched datasets describing the response of invertebrates, macrophytes, diatoms and fish to sediment chemistry were analysed for the elements antimony, arsenic, cadmium, chromium, copper, iron, lead, nickel, silver, tin and zinc.

Confidence intervals for each parameter were determined by bootstrapping. Here, distributions of model parameters were estimated by implementing 200 resamples of the observed dataset, each of which is obtained by random sampling with replacement from the original dataset (with each resample producing a dataset of equal size to the observed dataset). Bootstrapping is recommended when the theoretical distribution of a statistic of interest is complicated or unknown. Since the bootstrapping procedure is distribution-independent it provides an indirect method to assess the properties of the distribution underlying the sample and the parameters of interest that are derived from this distribution. Due to the shape of the curve being modelled here, this approach has a tendency to produce wide (and not normally distributed) confidence intervals, dependent upon how the upper range of sediment metal concentrations (above the threshold, b in Figure 3.1) is included within the resampling. Confidence intervals can also be estimated through a jack-knife procedure, a linear approximation of the bootstrap produced by systematically leaving out each observation from a dataset and calculating the estimate and then establishing the distribution of these estimates. Jack-knifing produces more normally distributed parameter estimates and smaller confidence intervals, but only the influence of each individual point is being considered. As more iterations are required to resample the full dataset as required by the jack-knife procedure, considerably more computing time is required compared with the

bootstrap procedure. A compromise between these two approaches was tested where the resampling procedure used in the bootstrap was structured to ensure that the resampling included data from across the whole range of sediment metal concentrations in the dataset. Here the range of sediment metal concentrations was divided into 5 equal sized bins, and the bootstrap resampling constrained such that each bin was sampled in proportion to the number of sites within the bin. Whilst the performance of the structured bootstrap was better using an artificial test dataset, similar non-normal confidence intervals were produced when the matched biology – chemistry datasets were used and it was decided that the additional computing time required did not justify the structured approach.

3.2 Results

The number of matched sites varied among BQEs with the highest number of matched sites for fish and invertebrates. Also, within BQEs the number of matched sites varied among the different metals, with mercury and silver typically having the least number of matched sites. There were also differences in the number of matched sites within each BQE, dependent upon which metric was used. As a consequence of these differences in the sizes of the datasets, for each metal, the range of sediment concentrations used, and the density of data across the range, varied with BQE. As quantile regression is sensitive to the density distribution of the data used, particularly with the threshold model used here, the variation in the size of data sets and range of sediment concentrations may have influenced the likelihood of detecting a threshold across the range of sediment metal – BQE combinations.

For fish, the EQR for individual species (as obtained from FCS2) was explored as a potential response variable, initially focussing on salmon (*Salmo salar* L.) and brown trout (*Salmo trutta* L.) as the most likely species to be found in mining areas. However, it became apparent that the probabilistic assumptions of the FCS2 tool resulted in data that did not fully satisfy the assumptions of the quantile regression threshold model. FCS2 produces a value for individual species, termed the EQR for that species, which represents the probability that the catch is equal to or greater than the expected catch for that species: if the expected catch is 0 for that species the EQR is 1. Thus, sites with high sediment metal concentrations returned high scores if they had a low probability of that species occurring there. Whilst the results for brown trout were more encouraging, those for salmon (which overall has a lower probability of occurrence) were difficult to interpret. The EQR for fish for the site as a whole is a compilation of the “EQR” values for individual species, is not affected in the same way and is a true EQR value.

Overall there appeared to be considerable differences in sensitivity across the different BQEs (Table 3.1). Across the suite of elements tested very few thresholds were detected for macrophytes, but all elements produced fits to the threshold model that were optimal for the number of taxa of invertebrates. Based on the number of taxa (and including those threshold values where the fit was close to optimal), the rank order from most sensitive to least sensitive was diatoms > invertebrates > fish > macrophytes. Diatoms had the lowest threshold values (with optimal or near optimal fits) for seven metals (cadmium, chromium, copper, iron, nickel, antimony and tin) and invertebrates for four metals (silver, arsenic, lead and zinc), again based on the number of taxa; macrophytes and fish were not lowest for any element. When other metrics were considered, diatoms had the lowest threshold for seven elements, invertebrates had three and fish one (Table 3.2). Whilst the diatom metrics % motile and TDI (Trophic Diatom Index – based on nutrient affinity) are relevant to the

functioning of the diatom assemblage and the latter to WFD classification, variation in these metrics may be influenced by factors other than the toxic effects of metals.

It should be noted, also, that the density of data and range of sediment metal concentrations varied across the different BQEs which will have influenced the likelihood of detecting a significant threshold.

For both invertebrates and diatoms the number of taxa provide a better response to sediment metal concentrations than did the other metrics tested (ASPT for invertebrates, and TDI and % motile taxa). This is not surprising as ASPT was developed as an index of sensitivity to organic pollution and TDI as an index of sensitivity to nutrient pollution, whereas the number of taxa is likely to indicate loss of those species that are sensitive to metals from the community.

With the exception of fish, surprisingly few thresholds were detected when the condition of sites was calculated as an ecological quality ratio (EQR). For invertebrates this is likely to be a consequence of fewer data (maximum 963 matched sites) being available for EQR than for NTAXA and ASPT. For diatoms and macrophytes it may be because EQRs are heavily weighted towards detection of nutrient stress: for diatoms the calculation of EQR is based entirely on TDI and does not involve the number of taxa present (which here appeared to be sensitive to sediment metal concentrations).

It was also apparent that for diatom EQR, macrophyte EQR and invertebrate ASPT EQR there were positive threshold relationships with sediment metal concentrations (i.e. response values increased once a threshold sediment metal concentration had been exceeded: Table 3.3.). It is possible that the areas around abandoned metal mines have not been subject to the same development and/or agricultural intensification that could influence the metrics behind these EQR values, i.e. through organic pollution or increased nutrient concentrations. However, it is likely that these metrics are confounded by the effects of metal contamination. These metrics could be affected if certain parts of the biological community are more sensitive to metal contamination, and thus affected preferentially. For diatoms, if the rapidly growing diatom taxa characteristic of higher nutrient conditions, which typically have mechanisms to facilitate rapid uptake of ions, are more sensitive to metal contamination and, therefore, are lost from the community, TDI scores would drop and EQR increase at high metal concentrations. Similarly for macrophytes, high metal concentrations may supress rapidly growing competitive taxa typical of higher nutrient conditions (reducing River Macrophyte Nutrient Index scores), increase species richness (Table 3.3) and, therefore, result in an apparent increase in EQR based on these two metrics. For invertebrates, the taxa with low ASPT scores are often sediment dwelling which could increase their susceptibility to sediment metal contamination: if these taxa are lost from the community ASPT would increase. The fish tool, FCS2, does not use metrics rather EQR is based on catch returns compared with expected catch.

Table 3.1 Summary of modelled threshold sediment metal concentrations (mg Kg^{-1}) from quantile regression.

	Canada° Interim sediment quality guidelines	Australia and New Zealand* Low trigger value	Invertebrates				Diatoms				Macrophytes				Fish			
			NTAXA	ASPT	EQR NTAXA	EQR ASPT	No.Taxa	% Motile	TDI	EQR	Plant Richness	No. Aquatic Taxa	RMNI	EQR	Site EQR	Salmon EQR	Trount EQR	No. Species
Cadmium	0.6	1.5	14.8		8.7		4	3.1							12		9.6	5.4
Chromium	37.3	80	391				182								128		261	
Copper	35.7	65	99		98		75		32.6			104						
Iron							41,404											
Lead	35	50	49.6				2,617	399							2,354			198
Nickel		21	202				41.4								169	170	197	156
Silver		1	7.9													49.6	51	
Tin			18.8				9		326		31				62.7	338		
Zinc	123	200	286				1043	382	153									2053
Antimony		2	13.7				1.8	9							55.5	65	85	
Arsenic	5.9	20	27				46.6								104		46	168

Threshold sediment metal concentrations for the biological quality elements and indices tested (parameter b in Figure 3.1) together with threshold environmental standards for Canada (° Canadian Council of Ministers of the Environment, 1999), and Australia and New Zealand (* ANZECC and ARMCANZ, 2000). Figures shown are from model fits that were optimal based on AIC criteria, figures in red are from models that were close to optimal. Details were not included, even if model fits were optimal, where the biological response did not fit expectations, i.e. the slope of the response above the threshold (parameter c in Figure 3.1) was positive. Full details of the modelled relationships are available in Appendix 2.

Table 3.2 Compiled results from quantile regression showing the geometric mean of threshold values and the lowest threshold value based on number of taxa (from optimal models), and the lowest of all metrics for each element.

	Canada° Interim sediment quality guidelines	Australia and New Zealand* Low Trigger Value (10% probability of effect)	Geometric mean based on number of taxa	Lowest based on number of taxa	Lowest of all metrics	
Cadmium	0.6	1.5	6.8	4	3.1	Diatom % motile
Chromium	37.3	80	267	182	128	Fish EQR
Copper	35.7	65	86.2	75	32.6	Diatom TDI
Iron			41,404	41,404	41,404	Diatom No. Taxa
Lead	35	50	295	49.6	49.6	Invertebrate NTAXA
Nickel		21	109	41.4	41.4	Diatom No. Taxa
Silver		1	7.9	7.9	7.9	Invertebrate NTAXA
Tin			17.4	9	9	Diatom TDI
Zinc	123	200	849	286	153	Diatom TDI
Antimony		2	5	1.8	1.8	Diatom No. Taxa
Arsenic	5.9	20	59.6	27	27	Invertebrate NTAXA

° Canadian Council of Ministers of the Environment (1999).
* ANZECC and ARMCANZ (2000).

Table 3.3 Summary of modelled threshold sediment metal concentrations (mg Kg^{-1}) from quantile regression where the biological response did not fit expectations, i.e. the slope of the response above the threshold (parameter c in Figure 3.1) was positive.

	Invertebrates				Diatoms				Macrophytes				Fish			
	NTAXA	ASPT	EQR NTAXA	EQR ASPT	No.Taxa	% Motile	TDI	EQR	Plant Richness	No. Aquatic Taxa	RMNI	EQR	Site EQR	Salmon EQR	Trount EQR	No. Species
Cadmium																
Chromium				*90*					*137*							
Copper				*251*												
Iron				*86,853*		*35,712*		*44,272*		*44,832*		*39,866*				
Lead									*1,700*							
Nickel																
Silver																
Tin																
Zinc																
Antimony																
Arsenic								*77.8*				*38.1*				

Figures shown are from model fits that were optimal based on AIC criteria, figures in red are from models that were close to optimal.

The values of modelled thresholds found here were compared with the Canadian interim sediment quality guidelines (Canadian Council of Ministers of the Environment, 1999, 1995) and the Australian and New Zealand Low Trigger Value (ANZECC and ARMCANZ, 2000) which are both largely based on laboratory trials (Table 3.1).

It was apparent that for copper the existing Canadian, and Australian and New Zealand sediment threshold environmental standards are comparable to the findings here, with threshold responses at relatively consistent concentrations across invertebrates (NTAXA, EQR NTAXA), diatoms (No. Taxa, TDI) and macrophytes (No. Aquatic Taxa).

A particular sensitivity to tin was noted across all BQEs (invertebrate NTAXA 18.8 mg Kg^{-1}, diatom No. taxa 20 mg Kg^{-1}, macrophyte plant richness 31 mg Kg^{-1}, fish site EQR 62.7 mg Kg^{-1}). The Canadian and the Australian and New Zealand sediment quality guidelines do not currently include values for tin.

For other elements, threshold concentrations varied over the different BQEs, with the response for diatoms similar to existing environmental standards for nickel, silver and antimony, and (accepting close to optimal models) for cadmium and arsenic. For invertebrates (NTAXA) the threshold concentrations for lead, arsenic and zinc were close to existing environmental standards. For fish and invertebrates (other metals), the modelled thresholds were typically an order of magnitude greater than existing sediment quality guidelines. Despite the uncertainty involved in the data matching exercise used to produce the datasets, these findings based on field data suggest that several of the existing sediment quality guidelines may be too precautionary, at least for fish and invertebrates.

4 Targeted Field Data Relating Bioavailable Metal Exposure to Community Response

Objective 3a

To undertake targeted field data collection to allow the measurement of bioavailable metal exposure in terms of the concentration bioaccumulated into the tissues of widespread benthic biomonitoring species, calibrate these measures of bioavailable metal against community response data.

4.1 Identification of field sites.

Informed by the collation of existing data undertaken in WP1b and consultation with EA staff involved with previous reviews (Environment Agency 2012a, b) 20 spatially-independent, replicate river catchments have been identified from across England and Wales in areas affected by metal mining facilities. Within each catchment, five monitoring sites have been selected (see Figure 4.1). The five sites include:

- an upstream control site,
- a site immediately downstream of the impacted stream reach,
- an additional control site on a comparable adjacent unimpacted watercourse,
- a site on the impacted stream further downstream in an erosional reach,
- and a site further downstream on the impacted stream along a depositional reach (but staying upstream of major urban areas).

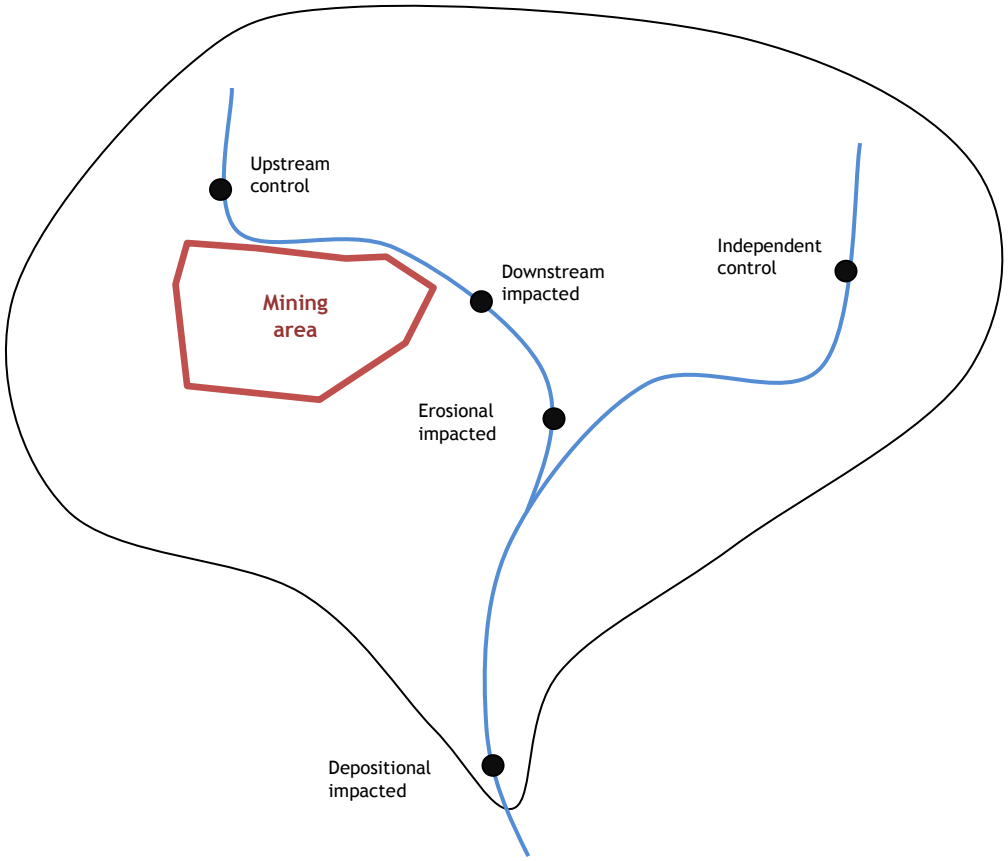


Figure 4.1. Schematic diagram of the arrangement of monitoring sites within each replicate catchment.

Catchments have been selected where mining is the dominant stressor on the system, avoiding catchments with large urban areas or other serious water quality issues that would confound our ability to link the biological response to the extent of metal-mining contamination.

To ensure that 20 catchments can be sampled an extended list of potential sites was drawn up from which the 20 would be selected (Table 4.1). The extended list was drawn up in case on arrival at a site it was clear that field conditions made it unsuitable to collect the samples required. The mining facilities associated with each catchment are given in Table 4.2.

4.2 Field sampling and laboratory methods

20 catchments have been sampled (see Figure 4.2 and Table 4.1). At each site four different types of data were collected:

- a sample of the macroinvertebrate community,
- biomonitor specimens for metal bioaccumulation analysis,
- a quantitative estimate of the amount of fine sediment in the stream bed, and
- a sample of the stream bed fine sediment for metals content, organic carbon, iron oxide, pH and particle size analysis.

4.2.1 Biological community sampling

At each site the benthic macroinvertebrate community was sampled using the standard UK WFD monitoring technique (3-minute kick sample plus 1 minute hand search) where all in-stream habitats were sampled in proportion to their occurrence in the sample reach (Murray-Bligh et al., 1997). The standard range of environmental variables were recorded either at the site (stream width and depth, velocity, substrate composition, pH and conductivity), or from map-based data (discharge category, altitude, distance from source and slope; Murray-Bligh et al., 1997). In addition photographs of the site were taken. Macroinvertebrate samples were fixed with dilute formalin and returned to the laboratory for subsequent identification and quantification to the lowest practicable taxonomic level, usually species or genus.

4.2.2 Biomonitor species sampling

A separate sample of >20 individuals from the biomonitor groups (Hydropsychidae, Rhycophilidae, Baetidae, Leuctridae and Gammaridae) have been collected from each site (as appropriate). Kick samples (additional to those outlined in 4.2.1) were collected until sufficient individuals found, and individuals picked and bagged in the field. Larger individuals were collected in preference to smaller individuals to ensure enough material for metal analysis. As a rule of thumb each bag contained the equivalent biomass of late instar Hydropsychidae; where only early instar individuals were present at a site then each bag was filled with sufficient small individuals to give the equivalent biomass of a late instar specimen.

By using common and ubiquitous biomonitor species we ensured that we had a measure of bioavailable metal from all sites.

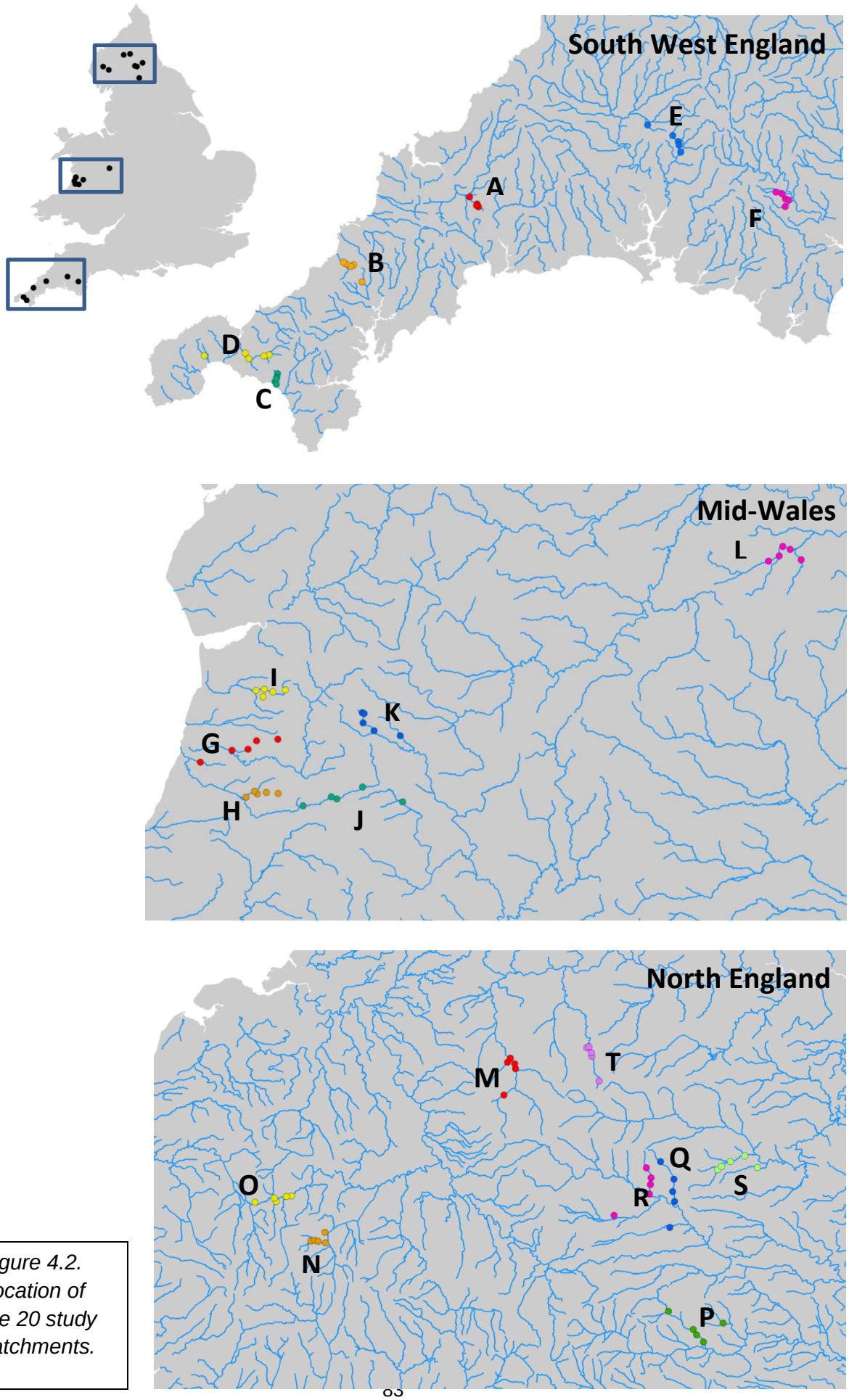


Figure 4.2. Location of the 20 study catchments.

Table 4.1 Field sites, grouped by catchment, with prior information on sediment metal concentrations (where available).

	Catchment	River_Name	Sampling_Site	EA_WB_ID	As	Cu	Fe	Zn
South West	A	Halgavor Stream	Independent_Control	GB108049000040				
		St. Lawrence Stream	Downstream	GB108049000040		H		H
		St. Lawrence Stream	Downstream_Erosional	GB108049000040		H		H
		St. Lawrence Stream	Downstream_Depositional	GB108049000040		H		H
	B	Bolingey Stream	Upstream_Control	GB108049000700		H		M
		Nanteague Stream	Independent_Control	GB108048002360				
		Bolingey Stream	Downstream	GB108049000700		H		M
		Bolingey Stream	Downstream_Erosional	GB108049000700		H		M
		Bolingey Stream	Downstream_Depositional	GB108049000700		H		M
	C	Porthleven Stream	Upstream_Control	GB108048002060				
		Tregew Stream	Independent_Control	GB108048002060				
		Porthleven Stream	Downstream	GB108048002060				
		Porthleven Stream	Downstream_Erosional	GB108048002060				
		Porthleven Stream	Downstream_Depositional	GB108048002060				
	D	Hayle	Upstream_Control	GB108049000380		M		M
		Trevaylor Stream	Independent_Control	GB108048002100		H		H
		Hayle	Downstream	GB108049000380		M		M
		Hayle	Downstream_Erosional	GB108049000380		M		M
		Hayle	Downstream_Depositional	GB108049000380		M		M
	E	River Burn	Upstream_Control	GB108047007880		M		M
		Quither Brook	Independent_Control	GB108047007731		H		H
		River Burn	Downstream	GB108047007880		M		M
		River Burn	Downstream_Erosional	GB108047007880		M		M
		River Burn	Downstream_Depositional	GB108047007880		M		M
	F	Mardle	Upstream_Control	GB108046005220				
		Dean Burn	Independent_Control	GB108046005190				
		Mardle	Downstream	GB108046005220				
		Mardle	Downstream_Erosional	GB108046005220				
		Mardle	Downstream_Depositional	GB108046005220				
Wales and Shropshire	G	Afon Melindwr	Upstream_Control	GB110063041590		M		M
		Penbryn	Independent_Control	GB110063041590				
		Afon Melindwr	Downstream	GB110063041590		M		M
		Afon Melindwr	Downstream_Erosional	GB110063041590		M		M
		Afon Melindwr	Downstream_Depositional	GB110063041590		M		M
	H	Nant Gwyn	Upstream_Control	GB110063041680				
		Nant Magwr	Independent_Control	GB110063041680		M		M
		Nant Cwmnewydion	Downstream	GB110063041680				
		Nant Cwmnewydion	Downstream_Erosional	GB110063041680				
		Nant Magwr	Downstream_Depositional	GB110063041680		M		M
	I	Afon Cyneiniog	Upstream_Control	GB110064043581				
		Nant Cwmere	Independent_Control	GB110064043581				
		Afon Cyneiniog	Downstream	GB110064043581				
		Afon Cyneiniog	Downstream_Erosional	GB110064043581				
		Afon Cyneiniog	Downstream_Depositional	GB110064043581				
	J	Afon Ystwyth	Upstream_Control	GB110063041720		M		M
		Afon Elan	Independent_Control	GB109055042300		H		H
		Afon Ystwyth	Downstream	GB110063041720		M		M
		Afon Ystwyth	Downstream_Erosional	GB110063041720		M		M
		Afon Ystwyth	Downstream_Depositional	GB110063041720		M		M
	K	Wye	Upstream_Control	GB109055042360		M		M
		Afon Bidno	Independent_Control	GB109055042340		H		H
		Wye	Downstream	GB109055042360		M		M
		Wye	Downstream_Erosional	GB109055042360		M		M
		Wye	Downstream_Depositional	GB109055042360		M		M
	L	Rea Brook	Upstream_Control	GB109054049540		H		H
		Pontesford Brook	Independent_Control	GB109054049500				
		Rea Brook	Downstream	GB109054049570		H		M
		Rea Brook	Downstream_Erosional	GB109054049570		H		M
		Rea Brook	Downstream_Depositional	GB109054049570		H		M

North East and North West	**M**	Black Burn	Upstream_Control	GB103023075410		H		H
		Gilderdale Burn	Independent_Control	GB103023075430				
		River South Tyne	Downstream	GB103023075530		H	H	H
		River South Tyne	Downstream_Erosional	GB103023075530		H	H	H
		River South Tyne	Downstream_Depositional	GB103023075530		H	H	H
	N	Red Tarn Beck	Upstream_Control	GB102076070740				
		Glencoyne Beck	Independent_Control	GB102076071020				
		Red Tarn Beck	Downstream	GB102076070740				
		Red Tarn Beck	Downstream_Erosional	GB102076070740				
		Red Tarn Beck	Downstream_Depositional	GB102076070740				
	O	River Glendermackin	Upstream_Control	GB112075070460		M		H
		Naddle Beck	Independent_Control	GB112075070420				
		River Glendermackin	Downstream	GB112075070460		M		H
		River Greta	Downstream_Erosional	GB112075073561		M	H	M
		River Greta	Downstream_Depositional	GB112075073561		M	H	M
	P	Arkle Beck	Upstream_Control	GB104027069170				
		Marske Beck	Independent_Control	GB104027069140				
		Arkle Beck	Downstream	GB104027069170				
		Arkle Beck	Downstream_Erosional	GB104027069170				
		Arkle Beck	Downstream_Depositional	GB104027069170				
	Q	Great Eggleshope Beck	Upstream_Control	GB103025072490		H		M
		Hindon Beck	Independent_Control	GB103024072690		H		H
		Eggleston Burn	Downstream	GB103025072490		H		M
		Eggleston Burn	Downstream_Erosional	GB103025072490		H		M
		Eggleston Burn	Downstream_Depositional	GB103025072490		H		M
	R	Hudeshope Beck	Upstream_Control	GB103025072480				
		Hargill Beck	Independent_Control					
		Hudeshope Beck	Downstream	GB103025072480				
		Hudeshope Beck	Downstream_Erosional	GB103025072480				
		Hudeshope Beck	Downstream_Depositional	GB103025072480				
	S	Spurlswood Beck	Upstream_Control	GB103024072700				
		Linburn Beck	Independent_Control	GB103024072720				
		Bedburn Beck	Downstream	GB103024072710				
		Bedburn Beck	Downstream_Erosional	GB103024072750				
		Bedburn Beck	Downstream_Depositional	GB103024072760		H		M
	T	River East Allen	Upstream_Control	GB103023074710		H		M
		Knockshield Burn	Independent_Control	GB103023074710				
		River East Allen	Downstream	GB103023074710		H		M
		River East Allen	Downstream_Erosional	GB103023074710		H		M
		River East Allen	Downstream_Depositional	GB103023074710		H		M

Table 4.2 Mining facilities in the catchments used as field sites.

Catchment	URN8	Site_name	Country	Local_authority
A		Tretoil Mine	England	Cornwall
B	1294	West Chiverton Mine	England	Cornwall
B	1300	Anna Mine	England	Cornwall
C	1295	Great Fortune Mine	England	Cornwall
C	1296	Metal Mine	England	Cornwall
D	1297	Godolphin Bridge Mine	England	Cornwall
E		West Wheal Friendship Tin Mine	England	Cornwall
F	1283	Brookwood Mine	England	South Hams District
G	1086	Cwmbrwyno	Wales	Ceredigion
G	1107	Goginan	Wales	Ceredigion
H	1115	Wemyss	Wales	Ceredigion
I	1123	Bwlchglas	Wales	Ceredigion
I	1125	Hafan	Wales	Ceredigion
J	1098	Cwmystwyth	Wales	Ceredigion
K	1109	Nantiago	Wales	Powys
L	1149	Roman Gravels	England	Shropshire
L	1151	Tankerville Mine	England	Shropshire
L	1155	Snailbeach	England	Shropshire
L	1161	Roundhill Mine	England	Shropshire
L	1164	Snailbeach Smelter	England	Shropshire
M	1005	Nenthead Mines	England	Eden District
M	1038	Holyfield Mine	England	Eden District
M	1043	Bentyfield Mine	England	Eden District
M	1059	White Sikes	England	Eden District
M	1072	Brown Gill, Garrigill	England	Eden District
M	1137	Hudgill Burn	England	Eden District
N	1257	Greenside Mine upper wastes	England	Eden District
N	1258	Greenside Mine lower wastes	England	Eden District
N	1259	Greenside Mine Tailings Dams	England	Eden District
O	1316	Gategill Mine	England	Eden District
P	1230	Windegg South Mine	England	Richmondshire District
P	1232	Sleigill Lead Mine	England	Richmondshire District
P	1233	Black Mine	England	Richmondshire District
P	1235	Dodgson Hush Mine	England	Richmondshire District
P	1236	Danby Lead Level Mine	England	Richmondshire District
Q	1027	California	England	County Durham
Q	1041	Wiregill	England	County Durham
Q	1053	Manor Gill	England	County Durham
R	1042	Lodge Sike	England	County Durham
R	1044	Coldberry	England	County Durham
S		Sharnberry Lead Mine	England	County Durham
T	1037	Mills Vein Level	England	Northumberland
T	1062	Swinhope Head Mine	England	Northumberland

All specimens were quickly rinsed in stream water to remove any attached material, individually bagged and frozen in the field in a portable freezer. Upon return to the laboratory the specimens were transferred to a -20 °C freezer.

In the laboratory, specimens were rinsed in double-distilled water and dried to constant mass in acid-washed Pyrex tubes. The specimens were then digested in Aristar grade concentrated nitric acid at 100 °C, made up to volume with double-distilled water and analysed for metals on a Vista-Pro CCD Simultaneous ICPOES. Accumulated metal concentrations were expressed as µg g^{-1} dry weight.

4.2.3 Quantitative fine sediment sampling

Immediately upstream of the RIVPACS and biomonitor sampling, 4 samples of deposited sediment mass were collected using the remobilisation technique working in an upstream direction, following the protocol of Duerdoth et al. (2015). Two samples are collected from depositional areas (patches with a propensity to deposit fine sediment e.g. eddies or areas of lower flow velocity such as pools or backwaters) and two from erosional areas (higher velocity areas in or close to the thalweg). At each position, the remobilisation stilling well was carefully lowered onto and pushed into the river bed to create a seal. The seal is important to prevent the winnowing of fines during the agitation process. The depth of water in the stilling well was measured and recorded, then one person stirred the water vigorously for 1 minute without making contact with the river bed. A sample of the resuspended surface drape of fine sediment was collected by drawing a vial through the water column. The first person then digs and stirs for 1 minute total (digs for 30 seconds then stirs for 30 seconds), aiming to dig to between approximately 10 and 20 cm depth if possible. A sample of the resuspended fine sediment (comprising both the surface drape of fine sediment and the fine sediment entrained within the river bed) is then collected by drawing a second vial through the water column.

The samples of fine sediment were refrigerated in cool boxes and returned to the laboratory within 5 days, where they were processed for dry mass and organic content (i.e. volatile solids following combustion at 550 °C). The masses were then converted to the deposited mass on the river bed from the concentration of resuspended fine sediment, the volume of water within the stilling well and the area of river bed sampled.

4.2.4 Fine sediment sampling for metals content & particle size analysis

Undisturbed areas accruing fine sediment were located visually within the same reach as used for the quantitative sediment sampling. At each position either the large stilling well or a smaller plastic version was placed over the fine sediment patch to isolate it from the flowing water and thus reduce the likelihood of loss of fines during removal of the sediment. The fine sediment was then scooped into N_2 filled pots, filling it right to the brim and ensuring that there were no obvious air pockets. This was done in a way that created as little disturbance to the fines as possible. While we were not seeking to maintain the integrity of any sediment layering, it was important to minimise the loss of very fine material during sampling. At each site five 100 ml samples and three 250 ml samples were collected.

These samples were then immediately placed in a portable freezer in the field and transferred to a -20 °C freezer upon return to the laboratory. Samples were kept at -20 °C until required, and then defrosted at room temperature. The samples were then oven dried at

40 °C over 1 week in (new) foil trays. Once dry, the samples were gently disaggregated in an agate pestle and mortar, which was wiped clean with acetone between samples. The samples were then sieved using 2 mm stainless steel test sieves. Sieves were cleaned in an ultrasonic bath and wiped with acetone between samples. The <2 mm and >2 mm factions were then weighed to 3 d.p. and stored in polyethene bags. The <2 mm fraction was used for further analysis.

a Subsampling: cone and quartering (*Adapted from Schumacher et al., 1990)*

Samples were tipped onto fresh baking parchment and subsampled in sections with a palette knife, using the cone and quarter technique to ensure the subsamples were representative of the whole sample. The palette knife was cleaned in acetone and the parchment replaced between each sample. 10 g, 1.5 g and 1 g subsamples were extracted for pH, particle size distribution and pseudo-total metal contents, respectively. The pH and metal samples were kept in 50 ml centrifuge tubes until required, and the particle size samples weighed directly into conical flasks for digestion. The weight of subsamples was recorded to 3 d.p. 10% of samples had triplicates taken for all three of the above analyses.

b pH *(Adapted from Rowell, 1996)*

25ml of deionised water (minimum 12 MΩcm) was added to the 10g of sediment in the centrifuge tube. Samples were agitated on a flatbed shaker on high speed for 15 minutes and then allowed to settle. Samples were analysed that day. Immediately prior to analysis each sample was shaken by hand for 10 seconds. A VWR pH100 meter was used to take readings. The meter was calibrated at the start of each day. Drift was checked hourly with pH 4 and 7 buffer solutions and the meter recalibrated if necessary. Readings were taken with the tip of the probe held 1-2cm above the settled sediment. Up to 10 minutes were given to allow readings to stabilise. Samples were analysed in batches according to river catchment in a randomised order.

c Particle size distribution

Removal of organic carbon (Adapted from US EPA methods (Schmacher, 2002)
10ml of H_2O_2 was added to each 1.5 g sample. Samples were left undisturbed at room temperature for 16 hours. 5 ml of H_2O_2 was added to each sample and then placed on a hotplate at 75 °C. A further 5 ml was added after 2, 4 and 6 hours. After 8 hours the hotplate was switched off. The following day samples were returned to the hotplate to evaporate off any remaining liquid. If the sample was still reacting with the H_2O_2 (indicated by effervescence) an additional 5 ml was added, and the sample left on the hotplate for a further 2 hours. This was repeated up to 3 more times if required. When the excess liquid had gone but samples were still moist they were removed from the hotplate and allowed to cool. The sediment was decanted directly into PSA tubes from the conical flasks using calgon (made by preparing 50 g sodium hexametaphosphate and 7 g anhydrous sodium carbonate in 1 L deionised water) and a disposable plastic spatula to get all of the sediment out of the flask. All plastics and glassware were washed in Decon-90 detergent for 24 hours prior to use.

Particle size distribution analysis
Within 2 days of digestion Samples were run on a Beckman coulter LS 12 300 laser granulometer. After a series of reproducibility tests, run conditions were optimised as follows;

Pump speed 100%
Obscuration fixed at 10% through auto-dilution
Run length 60 seconds including PIDS
Every sample run 3 times
30 seconds of sonication in the auto-prep station (power: 1)
5 seconds of sonication in the module before and between each of the 3 runs, (power: 1)
Samples were analysed in batches according to river catchment in a randomised order.

d Major and trace metal concentrations

Aqua Regia extraction (Adapted from Chen and Ma 2001)
Here all plastics and glassware washed in 10% HCL for 24 hours prior to use. Pre-weighed samples were emptied into 150ml conical flasks. Deionised water was used to rinse out any remaining sediment. Aqua regia was prepared fresh with 3:1 HCl:HNO_3 and 24ml was added to each sample (cold), and then capped with a reflux ball. Conical flasks were placed on a hotplate and brought up to 100 °C. After 3 hours samples were allowed to cool and then filtered through fluted Whatman (542) Ashless Hardened filter paper, through funnels into 50 ml volumetric flasks and made up to volume. Samples were decanted into centrifuge tubes for storage. For each batch, two aqua regia blanks and two certified reference materials (LGC river sediment 6187; LGC lake sediment SUD-1) were also run.

e Organic carbon

Inorganic carbon digestion: This method is an adaption of the method recommended in the instrument manual (Flash Elemental Analyser 1112 series).
Here all plastics and glassware were washed in 10% HCL for 24 hours prior to use. Glassware was then heated to 450 °C in a muffle furnace for 4 hours. 1 g of dry sediment taken randomly from sample bag using a spatula and dispensed into glass (pyrex) test tubes. Samples were moistened with a few drops of 25% HCl. Concentrated HCl was then added dropwise and observed for effervescence. Where the reaction was present, HCl was continually added to samples until reaction was complete. Samples were then dried in an oven at 40^{o}C (5-10 days). Using a glass rod, the samples were scraped out of the test tubes and into an agate pestle and mortar. Samples were crushed and then stored in foil envelopes in a desiccator until needed.

f Iron oxide

Oxalate extraction adapted from Philips and Lovley (1987)
Extraction reagent: ammonium oxalate (28gL-1) in oxalic acid (15gL-1), adjusted to pH 3. 100-150mg of crushed sample was weighed (4d.p.) into 50ml centrifuge tubes. 10ml of solution was pipetted onto each sample. Samples were agitated for 4 hours (Golden, 1994) on a flatbed shaker at high speed before being centrifuged at 4200 rpm for 20 minutes. The supernatant liquid was poured into 15ml centrifuge tubes and stored wrapped in foil at room temperature until analysis. Samples were washed prior to next extraction (van Oorschot and Dekkers, 2001).

Dithionite extraction adapted from Kostka and Luther (1994)
Extraction reagent: ammonium oxalate sodium dithionite (50gL-1) in 0.2M sodium citrate (58.82g/L).

10mL of extraction reagent was pipetted onto each rinsed sample. Each sample was then sealed and then placed in a water bath at 60^{o}C for 4 hours. Samples were removed and shaken by hand every 30 minutes. Samples were then centrifuged at 4200 rpm for 10 minutes. The supernatant liquid was poured into 15ml centrifuge tubes and stored at room temperature until analysis.

4.3 Results

4.3.1 Results of biomonitor analysis

Mean trace metal body burdens of the five taxa used as biomonitors are given in Tables 4.3 - 4.7. Arbitrary categories have been used to indicate elevated body burdens, indicating where there is high local bioavailability of that metal for that taxon. Differences between taxa will reflect differences in accumulation patterns. Because accumulated metal concentrations [C] in the insects typically follow a negative power relationship with dry weight [W], i.e. $[C] = a [W]^b$ where b is negative, concentrations derived from individuals of mean dry weight of 0.002 g or lower have been eliminated. Also, because of the relatively strong possibility of contamination of the odd sample with metal-rich sediment particles, any clear outliers have been eliminated.

Due to differences among the sites, no taxon was present at all the sties. The most complete dataset is for *Hydropsyche* spp. (Table 4.3) and that for *Gammarus* (Table 4.6) the most sparse.

Clear differences in metal bioavailabilites were apparent among the catchments. Given the occurrence of ores, it was expected that copper would be associated with arsenic, and zinc with lead. Zinc and lead may also co-occur with silver, and/or, less commonly with cadmium. These associations are clear in our data set. There was also considerable variation in the overall bioavailabilites between the catchments, with some catchments appearing to have relatively low bioavailability, either reflecting little contamination at these sites or that local conditions influence uptake of available metals. It is also worthy of note that some of the sites chosen as controls clearly had high local bioavailabilites of metals, either through unaccounted for mining facilities or local geology. In selecting sites every attempt was made to avoid inputs from mining facilities whilst keeping the geology similar to the contaminated sites.

It is logical that the strongest accumulators make the best biomonitors, offering a wider range of accumulated concentrations with which to distinguish between sites. Individual taxa may have atypically strong accumulation of a particular metal – for example *Rhyacophila* appears to have a strong affinity for silver. If we were just interested in silver, there would be almost no point in collecting the other taxa. However, as we are interested in a range of metals and a variety of environmental conditions (which influence which taxa are present) a suite of biomonitors (as used here) is the most informative approach.

Table 4.3 – 4.7. Trace metal body burden (as µg g^{-1} dry weight) of biomonitor species. High local bioavailability of that metal is indicated in red.

For *Hydropsyche* spp.

As – high >200, v high > 1,000, v v high >5,000:
Cd – high >15:
Cu – high >300, v high > 1,000:
Pb – high >100:
Zn – high >400; v high >1,000; v v high >10,000

For *Baetis* spp.

As – high >100, v high >1,000:
Cd – high >15:
Cu – high >100, v high >500:
Pb – high >100:
Zn – high >1,000, v high >5,000.

For *Leuctra* spp.

As – high >100, v high >1,000:
Cd – high >15, v high >50
Cu – high >100, v high > 1,000:
Pb – high > 50, v high > 500:
Zn – high >700, v high >2,000.

For *Gammarus* spp

As – high >75:
Cd – ?:
Cu – high >100:
Pb – high >100:
Zn – high >300; v high >1,000.

For *Rhyacophila* spp

Ag – high >10:
As – high >100, v high >1,000:
Cd – high >15, v high >100:
Cu – high >200:
Pb – high >50, v high >200:
Zn – high >500, v high >2,000.

Table 4.3 Trace metal body burden (as µg g^{-1} dry weight) of *Hydropsyche* spp. High local bioavailability of that metal for *Hydropsyche* spp is indicated in red. Blank cells indicate taxa missing from that site or metal below detection limits.

Catchment	Site	Ag	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	V	Zn
A St Lawrence Stream	Downstream	0.78	**417.0**	1.41	10.41	3.50	**1532**	6082	382	37.72	10.6	4.05	358
	Downstream erosional	1.48	**234.9**	1.72	19.32	8.67	**986**	7578	548	115.87	12.3	4.89	390
	Downstream depositional	0.63	4.4	1.86	14.12	2.71	82	1165	502	36.73	3.4	1.22	247
	Independent control	1.10	3.6	1.29	8.35	5.19	26	2770	225	61.38	4.6	2.12	187
B Bolingey Stream	Upstream Control	0.42	12.3	0.42	4.90	3.60	21	3005	749	29.31	3.5	2.55	144
	Downstream	0.37	47.0	4.96	4.66	2.64	112	3088	306	31.53	**197.4**	2.13	**1428**
	Downstream erosional	0.63	23.6	3.22	9.71	2.47	37	1868	494	62.89	57.4	1.03	**789**
	Downstream depositional	0.62	26.5	3.95	12.88	3.82	37	1466	514	109.99	75.1	0.97	**920**
	Independent control	0.38	26.2	1.15	18.56	2.48	17	1988	876	85.52	5.1	0.92	284
C Porthleven Stream	Upstream Control	0.30	**270.5**	1.08	6.19	3.08	57	1306	215	12.73	5.0	1.32	148
	Downstream	1.33	**11836.3**	13.91	3.13	4.90	**766**	6822	41	31.63	92.2	3.32	361
	Downstream erosional	0.90	**6152.1**	8.20	3.86	5.88	**646**	6902	74	21.69	62.6	4.81	383
	Downstream depositional	0.47	**9064.7**	11.37	4.36	4.93	**568**	7203	92	14.35	42.2	5.02	395
	Independent control	0.52	178.0	0.71	2.03	3.89	35	2497	128	18.40	6.3	2.74	172
D River Hayle	Upstream Control	1.31	**262.2**	3.78	2.65	6.17	298	1662	151	36.60	11.1	3.49	297
	Downstream												
	Downstream erosional	1.26	96.4	14.25	17.19	3.27	118	3455	252	39.01	29.4	3.17	**2200**
	Downstream depositional	1.08	133.8	**16.00**	16.70	5.94	194	3800	174	40.71	70.2	3.50	**2582**
	Independent control												
E River Burn	Upstream Control	0.73	9.7	0.79	0.96	2.93	18	797	147	16.54	7.1	1.44	190
	Downstream	1.01	19.8	1.14	1.01	2.11	14	354	86	8.27	9.9	1.01	343
	Downstream erosional	0.79	14.8	0.85	0.79	2.35	12	288	37	16.71	8.0	0.85	328
	Downstream depositional	0.99	16.1	0.99	0.99	2.42	24	389	85	12.31	9.6	0.99	**594**
	Independent control	0.47	6.4	0.47	0.80	1.25	4	340	70	7.88	2.5	0.47	67
F River Mardle	Upstream Control	2.85	2.9	2.85	2.85	2.85	20	906	132	25.50	3.3	2.85	169
	Downstream	2.77	33.9	2.77	3.31	4.68	53	1600	82	90.03	2.8	2.77	200
	Downstream erosional	1.50	12.0	1.50	1.98	2.72	50	1215	125	45.59	2.0	1.80	173
	Downstream depositional	1.86	9.0	1.86	3.37	5.73	53	1869	198	55.45	5.0	2.38	179
	Independent control	2.36	5.9	2.36	3.47	6.66	29	1782	300	76.99	2.9	2.90	169

Catchment	Site	Ag	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	V	Zn
G Afon Melindwr	Upstream Control	2.66	2.7	2.66	26.26	9.35	24	2113	133	303.05	76.2	2.66	197
	Downstream	1.33	3.1	1.33	3.61	5.11	28	1104	45	96.68	**118.4**	1.33	174
	Downstream erosional	0.99	4.2	1.12	1.22	2.23	23	1169	72	65.00	**167.3**	1.06	279
	Downstream depositional	1.17	2.2	1.23	1.96	5.55	24	2126	183	73.50	**210.1**	1.55	273
	Independent control	1.01	1.9	1.13	2.02	3.85	18	2586	390	81.78	14.3	1.67	137
H Nant Cwmnewydion	Upstream Control	1.40	1.4	1.40	2.12	1.62	18	1335	194	98.03	15.1	1.40	261
	Downstream	1.63	2.2	3.08	2.53	2.16	18	1041	93	106.95	**588.1**	1.63	**639**
	Downstream erosional	2.17	2.2	3.98	3.61	2.33	19	1462	71	105.11	**549.6**	2.17	**998**
	Downstream depositional	3.31	3.3	6.47	3.31	7.63	24	2518	81	164.46	**339.0**	3.31	**1333**
	Independent control	2.55	2.6	2.55	2.82	4.02	9	1621	286	41.99	3.3	2.55	227
I Afon Cyneiniog	Upstream Control	1.49	6.2	5.53	9.50	1.49	69	9166	217	78.30	21.1	1.71	**824**
	Downstream	1.17	5.2	1.27	5.25	2.04	56	6420	206	198.61	6.5	1.43	229
	Downstream erosional	1.12	47.2	1.12	3.81	8.51	50	9984	173	177.40	**146.5**	3.74	239
	Downstream depositional	0.96	11.3	4.70	1.46	7.32	31	4872	141	40.72	**128.3**	3.63	**742**
	Independent control	0.41	26.0	0.90	2.15	4.86	31	4888	134	50.62	72.8	3.73	152
J Afon Ystwyth	Upstream Control	1.14	2.5	1.14	1.63	1.92	19	2489	169	9.86	1.7	1.22	150
	Downstream	0.72	1.5	0.72	1.03	1.18	21	1499	92	4.08	**182.7**	0.72	226
	Downstream erosional	0.95	1.6	0.95	1.10	1.09	24	1415	99	8.51	**147.3**	0.95	246
	Downstream depositional	0.93	4.0	0.93	0.93	0.93	19	2202	162	11.31	**200.3**	0.93	256
	Independent control	0.68	4.0	0.68	0.68	0.68	16	3844	257	33.09	1.6	0.68	119
K River Wye	Upstream Control												
	Downstream												
	Downstream erosional												
	Downstream depositional												
	Independent control												
L Rea Brook	Upstream Control	1.83	11.7	1.83	3.01	7.34	33	1416	213	33.77	20.2	4.69	345
	Downstream	0.72	5.5	5.46	1.78	3.61	14	1187	146	13.21	88.8	2.41	**672**
	Downstream erosional	1.29	8.7	6.55	4.35	7.00	16	2384	895	16.40	**257.9**	6.21	**1233**
	Downstream depositional	0.87	6.1	5.02	3.43	7.78	17	3432	1351	22.10	**194.6**	6.02	**1050**
	Independent control	1.83	11.8	1.83	1.83	4.03	12	1636	319	18.42	9.9	2.89	174

Catchment	Site	Ag	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	V	Zn
M South Tyne	Upstream Control												
	Downstream												
	Downstream erosional	1.25	1.3	2.50	8.75	8.75	45	665	133	47.50	1.3	1.25	**494**
	Downstream depositional	0.59	0.6	5.35	5.35	0.59	44	992	195	50.50	49.3	1.19	**493**
	Independent control	0.37	0.4	6.55	7.52	0.66	35	1063	155	21.91	15.7	1.11	**592**
N Red Tarn Beck	Upstream Control												
	Downstream												
	Downstream erosional												
	Downstream depositional												
	Independent control	1.73	22.1	2.18	1.73	6.76	30	1370	150	121.76	48.1	1.73	**1397**
O River Greta	Upstream Control	0.97	39.3	0.97	4.33	2.12	10	911	470	18.52	4.5	1.31	142
	Downstream	0.51	28.3	2.24	3.17	1.86	16	1586	186	16.37	31.8	1.44	331
	Downstream erosional	1.42	47.3	1.42	2.87	3.59	10	1488	188	33.16	11.3	1.78	304
	Downstream depositional	1.26	23.5	1.49	2.80	2.08	7	1019	187	14.04	12.2	1.87	320
	Independent control	1.48	16.9	1.48	1.70	2.04	4	1766	422	9.59	1.9	2.25	136
P Arkle Beck	Upstream Control	1.02	7.8	2.95	1.13	1.89	53	1373	108	9.29	34.2	1.61	128
	Downstream	1.40	43.3	1.40	1.54	3.01	59	919	84	21.66	1.8	1.63	93
	Downstream erosional	1.24	112.0	**37.67**	9.57	5.93	110	2625	632	35.87	96.6	1.49	**16846**
	Downstream depositional	1.70	145.7	**48.79**	13.31	12.83	158	5886	788	49.33	**184.4**	7.35	**21205**
	Independent control	2.21	182.3	**50.79**	10.84	16.15	182	7331	827	85.77	**160.5**	5.42	**23137**
Q Eggleston Burn	Upstream Control												
	Downstream	1.43	1.6	1.59	1.43	1.43	9	2673	150	5.68	**492.1**	1.43	316
	Downstream erosional	1.87	2.0	1.87	1.87	3.47	9	1748	235	13.37	**259.2**	2.40	315
	Downstream depositional	1.26	1.4	1.26	1.26	2.05	9	1039	186	8.22	**233.3**	1.73	267
	Independent control	1.19	1.2	1.19	1.58	2.81	9	2356	417	9.01	7.9	1.96	130
R Hudeshope Beck	Upstream Control	0.84	0.8	0.84	0.84	0.84	13	2204	109	10.06	31.4	0.84	149
	Downstream	1.09	1.1	1.09	1.09	1.09	14	1587	188	27.45	**368.9**	1.09	172
	Downstream erosional	1.40	1.4	1.40	1.40	1.40	14	1555	219	9.49	**351.4**	1.40	300
	Downstream depositional	0.99	1.0	0.99	0.99	0.99	12	1315	185	5.19	**217.4**	0.99	186
	Independent control	2.50	2.5	2.50	2.50	2.50	17	1890	168	5.00	2.5	2.50	195

Catchment	Site	Ag	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	V	Zn
S Bedburn Beck	Upstream Control	2.26	2.3	2.26	2.90	3.37	15	2708	603	11.25	3.2	5.53	237
	Downstream	2.13	2.1	2.13	2.28	2.73	14	2602	353	6.31	66.6	3.65	334
	Downstream erosional	2.21	2.2	2.21	2.65	3.29	11	1800	345	6.37	46.6	2.65	277
	Downstream depositional	1.85	1.9	1.85	2.26	2.72	12	1633	265	6.63	56.3	2.00	257
	Independent control	1.27	1.3	1.27	3.12	4.51	18	2412	1218	16.40	8.8	1.95	155
T River East Allen	Upstream Control												
	Downstream	1.00	50.8	3.47	9.81	3.47	34	1473	274	55.48	3.0	1.42	**500**
	Downstream erosional	0.60	19.3	2.21	5.10	2.20	14	924	141	34.06	1.7	1.10	251
	Downstream depositional	0.59	44.2	3.56	9.50	2.06	33	1280	263	14.91	2.4	0.84	**467**
	Independent control	0.49	36.7	8.04	6.64	1.77	44	1166	130	17.40	6.0	0.89	**1817**

Table 4.4 Trace metal body burden (as µg g^{-1} dry weight) of *Baetis* spp. High local bioavailability of that metal for *Baetis* spp is indicated in red. Blank cells indicate taxa missing from that site or metal below detection limits.

Catchment	Site	Ag	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	V	Zn
A St Lawrence Stream	Downstream												
	Downstream erosional	0.29	66.0	**15.96**	88.57	1.55	**778**	1698	344	20.08	2.3	1.07	**2028**
	Downstream depositional	0.58	**112.0**	**33.90**	39.92	5.16	**264**	4950	674	41.77	5.9	4.10	**2046**
	Independent control	0.38	96.0	**19.05**	45.61	4.08	67	4788	308	15.40	5.9	3.69	**1570**
B Bolingey Stream	Upstream Control	0.31	17.0	2.67	7.69	6.31	37	3251	280	19.63	2.8	5.14	480
	Downstream	1.39	24.6	12.22	6.23	5.57	**134**	6998	192	153.54	**267.5**	4.18	**2909**
	Downstream erosional	0.77	53.8	**35.05**	16.58	5.75	**163**	6004	507	71.80	**151.5**	3.23	**8542**
	Downstream depositional	0.68	75.1	**34.62**	21.84	6.48	**140**	5114	296	56.49	**121.0**	3.58	**7668**
	Independent control	0.43	68.4	8.77	16.67	5.65	58	4814	471	46.64	8.5	3.57	**1820**
C Porthleven Stream	Upstream Control	0.23	**332.9**	11.79	33.54	5.92	**116**	2476	69	36.70	5.2	2.99	**1142**
	Downstream	0.65	**5734.0**	**18.80**	6.39	3.56	**776**	3331	32	23.68	40.4	2.82	**2290**
	Downstream erosional	0.45	**2518.9**	12.63	5.27	2.42	**497**	2508	32	12.73	21.1	2.21	**1677**
	Downstream depositional	0.61	**3462.6**	13.45	17.34	4.06	**437**	4731	62	68.04	17.9	3.42	**1334**
	Independent control	0.51	**165.8**	2.94	5.23	5.01	69	3529	97	29.45	5.7	4.78	400
D River Hayle	Upstream Control	1.30	**183.1**	2.60	1.84	4.50	**149**	501	34	43.84	2.6	1.30	392
	Downstream	0.41	29.0	0.55	0.58	1.22	13	93	17	10.96	0.7	0.45	134
	Downstream erosional	0.67	**108.5**	0.92	1.54	3.16	59	635	45	16.27	5.2	1.21	208
	Downstream depositional	1.03	**830.8**	1.41	3.04	5.48	**516**	5048	79	28.55	26.8	2.29	233
	Independent control	0.98	**1929.8**	2.46	4.40	6.15	**852**	7183	89	23.66	22.9	3.38	308
E River Burn	Upstream Control	0.13	7.7	0.22	1.73	1.69	6	495	307	9.54	1.1	1.03	41
	Downstream	0.24	26.3	0.51	0.28	1.56	36	446	36	9.76	0.4	0.78	32
	Downstream erosional	0.18	69.1	1.30	0.71	1.22	36	736	38	9.85	1.2	0.70	34
	Downstream depositional	0.27	52.1	3.63	2.91	2.21	49	863	126	14.51	1.8	1.35	68
	Independent control	0.33	24.6	0.83	0.91	4.12	82	1137	313	16.98	1.4	2.51	83
F River Mardle	Upstream Control	1.80	3.3	2.54	3.33	1.80	34	962	120	17.84	1.8	1.80	213
	Downstream	1.99	**124.0**	7.18	24.33	2.19	**108**	3627	195	18.51	4.2	1.99	515
	Downstream erosional	1.48	26.0	8.89	28.20	1.67	81	2376	191	14.98	3.2	1.99	481
	Downstream depositional	2.27	16.0	**18.28**	43.12	2.84	**127**	2475	135	32.91	3.6	2.27	**1027**
	Independent control	2.14	7.5	6.87	19.76	3.29	38	2509	131	49.74	2.4	2.49	400

Catchment	Site	Ag	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	V	Zn
G Afon Melindwr	Upstream Control	0.44	2.0	2.45	5.61	2.19	29	1503	123	20.35	34.8	1.28	231
	Downstream	0.13	2.3	2.77	1.00	0.82	29	447	15	5.57	31.7	0.51	843
	Downstream erosional	0.17	2.5	6.91	1.65	1.06	44	714	39	6.46	76.7	1.20	**1805**
	Downstream depositional	0.34	2.7	11.63	3.89	2.92	58	2111	99	18.24	**123.8**	1.86	**2407**
	Independent control	0.52	0.8	1.57	4.27	3.27	40	4412	258	8.86	7.4	3.22	477
H Nant Cwmnewydion	Upstream Control	0.96	2.8	1.14	6.81	8.05	32	7463	255	35.23	33.3	7.20	478
	Downstream	0.49	1.8	**23.29**	2.35	2.65	78	1465	100	27.76	**602.4**	0.89	**9473**
	Downstream erosional	0.53	1.9	**24.42**	2.01	3.90	71	2223	72	33.16	**620.6**	1.25	**10860**
	Downstream depositional	1.04	1.6	**17.02**	2.11	2.85	35	944	25	70.89	**100.6**	1.04	**8811**
	Independent control	1.54	2.4	2.17	5.88	7.03	33	4087	180	72.68	15.7	3.20	529
I Afon Cyneiniog	Upstream Control												
	Downstream												
	Downstream erosional	0.46	4.7	0.46	1.49	2.70	22	2761	113	16.04	9.4	2.68	132
	Downstream depositional	0.38	7.6	0.38	0.61	1.45	16	670	62	11.27	2.0	0.98	141
	Independent control												
J Afon Ystwyth	Upstream Control	0.79	14.3	0.79	6.74	5.50	25	1237	76	26.74	0.8	1.12	213
	Downstream	0.23	14.4	6.93	4.73	2.13	48	1436	65	11.46	65.2	1.74	**2285**
	Downstream erosional												
	Downstream depositional												
	Independent control												
K River Wye	Upstream Control												
	Downstream												
	Downstream erosional												
	Downstream depositional	0.50	11.6	0.50	0.55	0.98	13	402	16	17.61	15.5	0.50	199
	Independent control												
L Rea Brook	Upstream Control	0.40	2.5	1.67	2.96	7.10	28	2742	351	19.84	5.1	6.13	328
	Downstream	0.48	1.3	**17.09**	1.89	4.34	33	1852	87	33.83	53.7	2.18	**4049**
	Downstream erosional	0.75	1.8	**30.99**	2.59	5.88	46	2568	735	21.62	**158.0**	4.50	**8317**
	Downstream depositional	0.57	2.2	**31.79**	2.63	4.44	47	1995	733	20.12	**140.2**	2.96	**7350**
	Independent control	0.69	1.3	3.00	1.64	2.12	32	1338	242	26.85	2.1	1.28	282

Catchment	Site	Ag	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	V	Zn
M South Tyne	Upstream Control	0.29	3.4	1.40	3.85	1.71	19	2028	118	5.81	1.3	0.64	335
	Downstream	0.45	6.8	0.45	0.78	2.34	12	2417	86	9.54	31.6	1.93	135
	Downstream erosional	0.46	1.9	2.29	0.87	3.39	15	2343	71	22.12	72.5	1.87	384
	Downstream depositional	0.44	7.2	1.43	0.60	3.12	12	1352	79	11.47	36.0	0.91	289
	Independent control	0.21	3.7	0.21	0.21	1.19	8	384	36	4.21	0.4	0.31	73
N Red Tarn Beck	Upstream Control	0.20	0.5	0.34	0.99	0.68	7	351	39	9.60	13.0	0.48	55
	Downstream	0.23	1.7	0.90	0.49	1.81	9	300	22	9.59	98.4	0.36	56
	Downstream erosional	0.32	5.9	2.09	0.64	3.27	21	950	48	15.26	**114.7**	1.05	127
	Downstream depositional	0.36	1.6	0.36	1.96	3.28	9	416	45	18.17	16.2	0.47	57
	Independent control	0.19	0.2	0.33	0.42	1.18	3	58	4	6.76	13.7	0.23	50
O River Greta	Upstream Control	0.31	4.2	0.31	0.31	0.46	1	488	99	1.66	0.4	0.48	28
	Downstream	0.28	28.3	0.35	0.43	1.26	35	649	222	2.85	0.8	1.07	54
	Downstream erosional	0.28	52.2	0.97	0.94	1.24	19	200	22	6.18	0.9	0.37	530
	Downstream depositional	0.18	28.1	6.23	8.77	1.43	24	596	143	22.20	3.7	0.56	**2216**
	Independent control	0.47	19.0	2.08	2.93	3.38	19	1835	242	50.68	2.5	1.33	326
P Arkle Beck	Upstream Control	1.01	9.4	2.00	1.40	1.53	14	990	92	37.09	59.6	1.21	756
	Downstream	1.30	1.6	2.60	1.48	2.97	23	841	154	40.29	48.7	1.30	**1151**
	Downstream erosional	1.65	4.4	1.96	1.65	2.93	19	911	115	43.83	36.7	1.65	950
	Downstream depositional	0.84	6.5	0.84	0.84	2.16	11	573	70	23.18	7.0	0.94	495
	Independent control	0.90	3.4	1.34	0.90	1.58	12	463	47	29.70	22.4	0.90	395
Q Eggleston Burn	Upstream Control												
	Downstream	0.38	1.4	4.09	0.38	1.21	18	796	52	2.96	**131.7**	0.38	**1844**
	Downstream erosional	0.26	1.4	5.37	0.26	1.88	20	884	108	19.33	**134.6**	0.26	**2159**
	Downstream depositional	0.26	1.2	6.18	0.26	1.39	23	646	71	11.39	80.2	0.26	**1881**
	Independent control	0.29	0.8	1.34	0.29	2.13	22	1243	153	5.86	0.5	0.29	376
R Hudeshope Beck	Upstream Control	0.35	0.3	4.14	4.61	1.89	26	1306	120	5.68	23.3	0.35	823
	Downstream	0.23	0.2	9.78	1.54	2.18	21	1244	127	8.38	**231.5**	0.23	**1772**
	Downstream erosional	0.29	0.3	6.15	1.34	2.55	19	1235	77	4.54	82.1	0.29	**1558**
	Downstream depositional	0.32	0.3	9.66	1.75	2.20	22	1138	108	5.00	**169.0**	0.32	**2262**
	Independent control	0.30	0.3	3.69	2.06	1.79	14	1032	131	7.50	10.3	0.30	935

Catchment	Site	Ag	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	V	Zn
S Bedburn Beck	Upstream Control	0.62	0.6	6.40	6.45	0.62	25	1665	257	6.68	0.6	0.62	917
	Downstream	0.43	0.4	6.26	2.40	0.43	25	1391	141	6.75	37.1	0.43	**1805**
	Downstream erosional	0.38	0.4	6.50	3.22	0.38	24	1391	153	7.86	25.1	0.38	**1923**
	Downstream depositional	0.38	0.4	9.39	3.73	0.38	27	1518	195	9.47	23.6	0.38	**2227**
	Independent control	0.18	0.2	0.18	2.39	0.18	24	966	251	5.73	1.0	0.18	203
T River East Allen	Upstream Control												
	Downstream	0.18	3.6	0.46	0.25	1.12	9	555	46	4.75	24.8	0.41	126
	Downstream erosional	0.22	2.5	0.35	0.28	0.90	7	757	48	5.01	21.7	0.94	121
	Downstream depositional	0.22	1.7	0.39	0.44	0.86	6	877	63	5.40	26.1	0.79	129
	Independent control	0.38	1.1	0.38	0.60	1.21	6	1319	79	10.68	1.5	0.78	78

Table 4.5 Trace metal body burden (as µg g^{-1} dry weight) of *Leuctra* spp. High local bioavailability of that metal for *Leuctra* spp is indicated in red. Blank cells indicate taxa missing from that site or metal below detection limits.

Catchment	Site	Ag	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	V	Zn
A St Lawrence Stream	Downstream												
	Downstream erosional												
	Downstream depositional	1.84	**146.1**	2.32	9.98	11.89	**160**	6957	588	88.41	18.1	8.42	298
	Independent control	1.36	**117.8**	1.36	4.29	6.64	39	5729	152	31.26	9.0	4.69	213
B Bolingey Stream	Upstream Control												
	Downstream	1.14	62.5	**22.33**	17.45	9.90	**158**	8702	487	116.63	**306.6**	6.83	**2295**
	Downstream erosional	0.80	10.6	**16.43**	22.52	4.97	82	3692	433	108.40	**83.3**	3.02	**836**
	Downstream depositional	0.84	45.5	**16.44**	8.55	4.94	75	3389	591	55.66	**93.4**	4.66	**845**
	Independent control												
C Porthleven Stream	Upstream Control	0.73	**210.3**	4.19	2.51	4.79	80	2576	77	19.27	7.3	2.95	203
	Downstream	0.88	**4199.3**	9.29	5.84	6.12	**506**	4624	68	32.43	38.5	4.75	279
	Downstream erosional	0.93	**3519.5**	9.86	7.53	10.28	**527**	6748	134	44.23	48.8	8.85	351
	Downstream depositional												
	Independent control												
D River Hayle	Upstream Control												
	Downstream	1.14	**2331.3**	2.45	4.18	4.73	**674**	5965	156	34.32	27.6	3.28	308
	Downstream erosional	0.98	**3131.3**	5.15	6.08	5.76	**1031**	8028	266	39.31	20.5	3.57	512
	Downstream depositional												
	Independent control												
E River Burn	Upstream Control	0.84	36.3	**15.18**	6.69	3.35	66	1641	134	25.64	18.2	1.53	**4385**
	Downstream												
	Downstream erosional												
	Downstream depositional												
	Independent control												
F River Mardle	Upstream Control												
	Downstream												
	Downstream erosional												
	Downstream depositional												
	Independent control	2.00	2.0	2.00	2.00	2.00	48	1448	126	40.00	2.0	2.00	264

Catchment	Site	Ag	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	V	Zn
G Afon Melindwr	Upstream Control	0.70	0.7	2.11	2.08	4.46	56	4832	167	26.11	**172.3**	3.29	270
	Downstream	0.53	1.4	4.12	1.18	8.76	73	3474	73	32.33	**163.4**	2.64	269
	Downstream erosional	0.53	5.6	8.02	1.12	3.74	90	3382	98	20.51	**264.1**	2.64	338
	Downstream depositional	0.52	0.6	6.71	1.22	4.08	76	3109	92	21.21	**160.0**	2.35	321
	Independent control	0.55	1.4	0.55	2.86	8.67	54	7948	367	25.98	13.3	6.25	236
H Nant Cwmnewydion	Upstream Control	1.41	1.4	1.41	3.38	6.11	38	5693	271	93.36	25.6	3.08	277
	Downstream												
	Downstream erosional												
	Downstream depositional	2.18	3.0	14.19	2.66	6.78	41	4854	156	73.17	**374.8**	2.66	**1288**
	Independent control	1.52	1.5	1.52	2.23	5.43	41	4992	202	76.67	14.9	2.55	257
I Afon Cyneiniog	Upstream Control	1.62	20.7	1.62	1.62	2.82	31	757	100	51.10	13.8	1.62	689
	Downstream	1.34	5.6	1.34	1.34	1.91	33	834	81	44.66	25.6	1.34	**731**
	Downstream erosional	1.18	1.1	1.18	1.49	1.18	14	1203	104	39.86	1.2	1.18	207
	Downstream depositional	0.99	1.0	0.99	1.14	0.99	11	3246	77	26.98	**197.2**	0.99	140
	Independent control	0.76	2.1	0.76	2.70	0.76	18	3345	173	24.18	**147.9**	0.76	253
J Afon Ystwyth	Upstream Control												
	Downstream												
	Downstream erosional												
	Downstream depositional												
	Independent control												
K River Wye	Upstream Control	0.86	7.8	0.86	1.10	2.41	12	874	53	58.24	8.9	0.96	164
	Downstream	0.66	**124.0**	1.68	1.76	6.23	39	901	72	64.34	4.1	1.07	**847**
	Downstream erosional	2.37	**205.0**	**18.05**	14.56	14.75	**137**	5736	629	174.73	**69.8**	4.94	**6535**
	Downstream depositional	1.16	**123.6**	12.86	6.72	12.92	69	4735	260	164.89	**104.1**	5.36	**5317**
	Independent control	1.46	**157.5**	3.40	3.60	10.24	33	6419	241	229.87	**83.2**	3.76	**976**
L Rea Brook	Upstream Control												
	Downstream												
	Downstream erosional												
	Downstream depositional												
	Independent control	1.83	1.8	1.83	1.83	6.01	69	1942	221	103.97	3.4	2.52	205

Catchment	Site	Ag	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	V	Zn
M South Tyne	Upstream Control	0.96	18.1	1.37	1.10	3.00	27	675	74	71.27	27.1	1.07	**1348**
	Downstream	0.73	**490.4**	0.88	2.25	1.28	19	1988	231	37.96	40.6	1.66	346
	Downstream erosional	0.74	**612.2**	**20.57**	6.92	1.48	93	2250	438	38.21	40.9	2.62	**1306**
	Downstream depositional	0.87	**434.5**	**54.01**	4.17	1.74	**104**	2139	202	29.61	**540.1**	2.91	**3536**
	Independent control	0.62	**464.6**	**52.02**	2.66	1.51	93	1773	105	26.01	**611.5**	2.41	**3175**
N Red Tarn Beck	Upstream Control	0.91	0.9	1.23	1.38	6.69	19	226	24	33.52	**51.1**	0.91	280
	Downstream	1.19	1.2	1.19	1.19	6.79	30	573	81	43.03	48.6	1.37	345
	Downstream erosional	1.23	1.2	1.23	1.23	11.13	10	747	31	117.14	26.1	1.48	122
	Downstream depositional	0.66	1.3	0.66	0.77	4.57	16	332	19	32.24	29.8	0.77	112
	Independent control	1.12	21.6	12.53	4.39	5.13	20	2488	182	103.13	28.1	1.86	**1141**
O River Greta	Upstream Control	1.35	44.0	1.35	2.71	6.64	35	1370	248	227.53	6.1	1.35	211
	Downstream	0.81	18.9	1.65	1.62	3.27	42	1507	149	51.75	15.7	1.98	294
	Downstream erosional	0.87	45.2	0.87	2.61	5.22	30	2470	220	93.91	26.1	1.74	358
	Downstream depositional	0.82	29.9	1.62	2.20	5.07	33	1739	223	47.55	13.4	1.75	495
	Independent control	1.20	31.6	1.20	1.20	3.79	44	3583	586	11.24	3.8	5.36	233
P Arkle Beck	Upstream Control												
	Downstream												
	Downstream erosional												
	Downstream depositional	3.33	10.0	6.67	3.33	3.33	**123**	760	220	66.67	**326.7**	3.33	**2817**
	Independent control												
Q Eggleston Burn	Upstream Control	0.84	2.0	0.84	0.84	2.75	23	5529	122	11.45	7.0	0.84	159
	Downstream	0.58	1.1	1.02	0.76	3.14	19	2638	169	12.62	**326.8**	2.83	350
	Downstream erosional	0.65	0.7	0.92	0.80	3.13	19	2385	225	9.55	**256.9**	2.48	302
	Downstream depositional	1.05	1.5	1.61	1.05	1.05	17	1794	135	13.53	**180.0**	1.05	268
	Independent control	0.90	1.1	0.90	0.90	2.66	27	3600	357	7.79	3.3	0.90	163
R Hudeshope Beck	Upstream Control	0.94	0.9	0.94	0.94	6.22	22	3317	249	50.03	**55.8**	0.94	194
	Downstream	0.75	0.7	0.75	0.75	3.31	24	2918	174	4.76	**438.5**	0.75	243
	Downstream erosional	0.98	1.0	0.98	0.98	3.49	21	2436	161	4.55	**309.7**	0.98	410
	Downstream depositional	0.99	1.0	0.99	0.99	5.28	26	3159	132	6.40	**176.1**	0.99	247
	Independent control	0.82	0.8	0.82	0.82	3.94	15	2144	155	24.32	24.3	0.82	209

Catchment	Site	Ag	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	V	Zn
S Bedburn Beck	Upstream Control	0.49	0.5	0.49	0.49	0.49	29	2609	538	13.33	9.5	0.49	219
	Downstream	0.65	0.6	0.65	0.65	0.65	23	3204	488	39.70	**99.8**	0.65	372
	Downstream erosional	0.83	0.8	0.83	1.32	1.59	19	3096	485	49.21	**67.0**	1.42	333
	Downstream depositional	0.81	0.8	1.31	1.82	3.35	16	2959	345	21.62	**60.0**	2.50	251
	Independent control												
T River East Allen	Upstream Control	1.14	18.4	1.38	1.58	2.05	38	1539	429	36.87	34.0	1.32	**1166**
	Downstream	0.69	19.9	0.85	1.02	3.10	31	1093	178	43.08	3.7	0.69	419
	Downstream erosional	0.45	0.9	0.81	0.45	0.56	2	147	3	17.65	1.8	0.45	20
	Downstream depositional	0.83	6.3	0.89	1.89	2.14	22	379	169	18.45	1.1	0.97	200
	Independent control	1.09	**117.6**	1.09	5.52	7.62	32	4543	369	33.53	4.8	4.27	199

Table 4.6 Trace metal body burden (as µg g^{-1} dry weight) of *Gammarus* spp. High local bioavailability of that metal for *Gammarus* spp is indicated in red. Blank cells indicate taxa missing from that site or metal below detection limits.

Catchment	Site	Ag	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	V	Zn
A St Lawrence Stream	Downstream												
	Downstream erosional												
	Downstream depositional	0.94	41.3	3.92	6.71	3.77	**113**	1614	163	50.08	3.7	2.22	101
	Independent control	0.77	74.6	2.73	2.08	2.38	78	855	41	46.23	3.6	1.31	90
B Bolingey Stream	Upstream Control	0.18	28.8	0.47	1.03	3.43	78	1101	105	23.32	0.8	1.85	88
	Downstream												
	Downstream erosional												
	Downstream depositional												
	Independent control												
C Porthleven Stream	Upstream Control												
	Downstream												
	Downstream erosional												
	Downstream depositional												
	Independent control	0.22	**85.2**	1.74	0.73	2.59	64	1274	55	11.84	3.7	2.10	72
D River Hayle	Upstream Control												
	Downstream												
	Downstream erosional	0.88	0.9	0.88	0.88	0.88	23	1976	154	21.18	0.9	0.88	71
	Downstream depositional	1.00	37.5	1.00	2.34	2.31	23	4511	288	33.90	37.0	2.03	149
	Independent control	0.54	7.8	0.56	1.37	1.29	15	1760	120	15.56	37.1	1.58	134
E River Burn	Upstream Control	0.48	18.5	0.69	1.98	0.98	8	563	135	9.37	4.3	0.86	105
	Downstream	0.61	39.4	2.95	4.23	2.59	19	2110	288	21.71	46.3	1.69	**418**
	Downstream erosional	0.47	17.8	0.80	1.22	0.93	4	595	72	7.51	4.7	0.93	129
	Downstream depositional	0.42	9.3	0.49	1.02	1.01	2	368	66	12.89	4.4	0.48	94
	Independent control	0.27	4.6	0.32	0.65	0.46	2	208	51	2.85	1.4	0.42	60
F River Mardle	Upstream Control												
	Downstream												
	Downstream erosional	0.42	7.7	1.18	1.02	1.39	69	756	85	9.66	1.0	1.26	65
	Downstream depositional	0.65	8.4	1.42	1.25	2.42	**109**	985	113	28.88	1.3	0.99	115
	Independent control												

Catchment	Site	Ag	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	V	Zn
G Afon Melindwr	Upstream Control												
	Downstream												
	Downstream erosional												
	Downstream depositional												
	Independent control												
H Nant Cwmnewydion	Upstream Control												
	Downstream												
	Downstream erosional												
	Downstream depositional												
	Independent control												
I Afon Cyneiniog	Upstream Control												
	Downstream												
	Downstream erosional												
	Downstream depositional												
	Independent control												
J Afon Ystwyth	Upstream Control												
	Downstream												
	Downstream erosional												
	Downstream depositional												
	Independent control												
K River Wye	Upstream Control												
	Downstream												
	Downstream erosional												
	Downstream depositional												
	Independent control												
L Rea Brook	Upstream Control	0.21	9.3	0.22	0.58	1.94	75	940	323	4.77	2.6	1.98	80
	Downstream	0.24	7.6	7.59	0.64	2.58	68	1310	179	8.95	50.3	2.70	286
	Downstream erosional	0.32	8.0	7.41	0.80	3.38	77	1643	250	4.81	89.2	3.22	**330**
	Downstream depositional	0.30	8.8	6.94	0.67	3.62	73	1401	300	16.32	56.5	2.66	287
	Independent control	0.24	8.1	0.26	0.24	1.43	65	465	98	11.45	0.3	0.89	61

Catchment	Site	Ag	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	V	Zn
M South Tyne	Upstream Control												
	Downstream												
	Downstream erosional												
	Downstream depositional												
	Independent control												
N Red Tarn Beck	Upstream Control												
	Downstream												
	Downstream erosional	1.11	1.1	1.11	1.11	4.44	8	517	52	97.78	16.7	1.11	**328**
	Downstream depositional												
	Independent control	1.36	10.9	1.36	1.36	6.82	11	2523	74	114.55	38.2	1.36	**679**
O River Greta	Upstream Control												
	Downstream												
	Downstream erosional												
	Downstream depositional												
	Independent control	0.35	30.8	0.42	0.49	1.33	40	810	256	3.35	0.8	1.25	61
P Arkle Beck	Upstream Control	0.99	35.7	2.67	6.84	5.34	40	2145	257	22.07	1.9	1.34	**1096**
	Downstream	1.18	47.1	1.18	8.24	7.06	29	2788	214	28.24	1.2	2.35	**562**
	Downstream erosional	0.95	49.5	3.81	14.29	9.52	54	4876	409	31.43	3.8	4.76	**1190**
	Downstream depositional	0.57	3.9	0.97	2.70	3.18	15	3599	329	15.43	8.5	1.03	215
	Independent control	1.03	12.2	1.03	2.41	3.58	17	5959	568	14.80	19.7	1.93	189
Q Eggleston Burn	Upstream Control												
	Downstream												
	Downstream erosional												
	Downstream depositional												
	Independent control												
R Hudeshope Beck	Upstream Control												
	Downstream												
	Downstream erosional												
	Downstream depositional												
	Independent control												

Catchment	Site	Ag	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	V	Zn
S Bedburn Beck	Upstream Control	0.62	0.6	1.59	0.67	1.06	53	554	165	9.31	3.3	0.83	78
	Downstream												
	Downstream erosional												
	Downstream depositional												
	Independent control	0.85	0.9	0.85	0.85	2.15	55	663	128	21.48	5.2	0.85	65
T River East Allen	Upstream Control												
	Downstream												
	Downstream erosional												
	Downstream depositional												
	Independent control	0.15	**122.9**	0.15	1.61	1.98	24	1164	53	13.43	2.5	0.95	42

Table 4.7 Trace metal body burden (as µg g^{-1} dry weight) of *Rhyacophila* spp. High local bioavailability of that metal for *Rhyacophila* spp is indicated in red. Blank cells indicate taxa missing from that site or metal below detection limits.

Catchment	Site	Ag	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	V	Zn
A St Lawrence Stream	Downstream												
	Downstream erosional	0.86	**120.0**	1.49	4.71	0.86	**409**	3299	230	29.51	2.1	0.86	428
	Downstream depositional	0.59	9.1	1.65	4.86	0.84	50	697	215	21.02	1.4	0.75	282
	Independent control	0.46	6.5	0.61	1.05	1.07	18	492	38	18.50	1.3	0.46	195
B Bolingey Stream	Upstream Control												
	Downstream												
	Downstream erosional	1.34	1.3	3.58	2.28	2.17	26	518	108	60.74	12.0	1.34	**650**
	Downstream depositional	0.96	1.0	2.99	2.40	1.80	30	526	150	53.96	17.2	0.96	**552**
	Independent control	0.90	1.3	1.16	3.07	1.64	22	1325	227	73.78	2.4	1.21	360
C Porthleven Stream	Upstream Control	0.35	84.4	1.24	0.73	0.88	31	420	47	7.70	1.4	0.45	201
	Downstream												
	Downstream erosional												
	Downstream depositional												
	Independent control												
D River Hayle	Upstream Control												
	Downstream												
	Downstream erosional	2.14	**9219.9**	**17.45**	41.84	14.25	**2734**	22503	1089	64.20	**50.3**	11.13	**1774**
	Downstream depositional	1.06	**1280.3**	3.03	4.33	4.31	**365**	4993	107	25.59	13.4	1.62	281
	Independent control	0.73	36.0	0.84	0.94	2.48	16	691	52	15.06	2.7	1.03	87
E River Burn	Upstream Control	0.74	82.8	**19.17**	17.76	4.48	78	2191	348	54.88	16.2	2.13	**5707**
	Downstream	0.84	**111.4**	**24.90**	33.41	6.55	95	2612	564	93.90	16.8	2.36	**8548**
	Downstream erosional	0.82	30.2	2.62	3.51	5.31	34	2372	308	99.00	5.7	1.99	415
	Downstream depositional												
	Independent control	0.72	19.3	1.18	1.56	3.49	30	1368	132	74.81	14.2	1.56	273
F River Mardle	Upstream Control	1.73	2.3	1.73	1.73	1.73	17	296	122	31.80	2.8	1.73	305
	Downstream	2.97	3.0	2.97	2.97	2.97	15	83	27	14.85	3.0	2.97	264
	Downstream erosional	1.36	1.4	1.36	1.36	1.36	31	387	134	26.46	1.4	1.36	274
	Downstream depositional	1.81	2.4	1.81	1.81	1.81	19	219	84	23.68	3.0	1.81	266
	Independent control	2.09	2.1	2.09	2.09	2.09	17	385	114	19.24	3.1	2.09	300

Catchment	Site	Ag	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	V	Zn
G Afon Melindwr	Upstream Control	1.54	2.7	1.51	1.54	3.44	24	393	67	58.18	17.8	1.54	388
	Downstream	0.44	0.8	0.42	0.51	1.68	20	250	13	28.33	16.3	0.44	302
	Downstream erosional	0.75	0.8	1.11	0.87	2.19	26	311	18	69.90	22.7	0.75	397
	Downstream depositional	0.69	0.9	0.89	2.24	2.93	21	366	38	66.50	33.0	0.95	398
	Independent control	0.91	0.9	0.91	15.35	3.89	25	632	99	83.79	3.2	0.91	295
H Nant Cwmnewydion	Upstream Control	1.23	1.2	1.23	1.71	2.94	22	617	94	119.44	5.6	1.19	438
	Downstream	1.09	1.1	2.54	1.33	5.07	15	454	37	142.64	**68.4**	1.34	**1094**
	Downstream erosional	0.87	0.9	2.60	0.87	1.21	16	320	34	95.26	**78.1**	1.03	**1052**
	Downstream depositional	1.02	1.0	1.91	1.02	1.77	20	311	22	58.81	29.6	1.23	**1148**
	Independent control	1.37	1.4	1.37	1.37	2.09	18	425	69	43.13	6.7	1.37	336
I Afon Cyneiniog	Upstream Control	0.91	0.9	0.91	2.04	0.91	11	1928	167	50.21	25.9	0.91	179
	Downstream	1.22	1.2	1.22	2.47	1.22	21	3659	271	58.66	3.8	1.22	195
	Downstream erosional	1.46	2.0	1.46	3.69	1.46	20	1219	279	59.50	4.7	1.46	258
	Downstream depositional	**71.63**	**2394.0**	**260.63**	5.24	8.57	**1067**	1179	133	41.08	0.6	15.68	**1918**
	Independent control	1.42	1.1	11.25	11.40	3.75	42	4068	114	103.59	**67.4**	4.13	**606**
J Afon Ystwyth	Upstream Control	1.05	2.3	1.05	1.18	3.31	27	800	104	66.76	1.1	1.18	249
	Downstream	0.55	11.5	0.86	0.59	2.19	24	660	66	26.15	**98.5**	0.87	**673**
	Downstream erosional	1.19	5.8	1.19	1.19	4.59	31	618	101	86.42	**80.1**	1.19	**879**
	Downstream depositional	0.76	4.5	0.96	0.76	2.72	19	612	41	43.13	34.2	1.00	470
	Independent control	0.67	2.8	0.67	0.72	0.92	20	582	136	4.95	0.7	0.80	253
K River Wye	Upstream Control												
	Downstream	0.53	0.5	0.53	1.22	3.91	10	3166	69	69.77	20.4	1.35	114
	Downstream erosional	1.03	0.9	8.16	2.36	6.48	32	5940	168	68.70	**79.5**	2.84	**2935**
	Downstream depositional	0.82	0.8	11.15	2.98	4.35	33	4652	101	21.45	**62.8**	1.64	**4747**
	Independent control	0.52	0.5	7.83	2.09	2.09	25	2990	88	14.09	48.0	1.57	**3308**
L Rea Brook	Upstream Control	2.68	2.7	2.68	3.79	8.03	20	945	366	217.05	5.4	2.68	311
	Downstream	1.10	1.2	2.48	1.26	5.69	20	1133	382	102.55	17.9	1.66	**993**
	Downstream erosional	1.83	1.8	3.66	1.83	8.02	23	1242	205	199.22	8.6	1.83	**1074**
	Downstream depositional	0.67	0.7	1.20	0.67	2.40	19	625	309	57.42	16.6	0.75	**635**
	Independent control	1.56	2.7	1.56	1.56	5.42	17	744	222	129.10	3.1	1.56	249

Catchment	Site	Ag	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	V	Zn
M South Tyne	Upstream Control	0.32		**15.57**	0.32	0.97	29	859	49	24.00	**314.3**	1.62	**1171**
	Downstream												
	Downstream erosional	0.34	0.3	**34.66**	1.67	1.00	52	1664	85	19.92	**407.2**	2.61	**2001**
	Downstream depositional	0.48	0.5	**29.82**	3.47	0.78	43	1640	95	36.88	**377.5**	1.98	**1775**
	Independent control												
N Red Tarn Beck	Upstream Control	0.63	8.8	8.34	0.89	3.56	7	815	44	79.91	40.0	0.63	**511**
	Downstream	0.97	4.8	5.58	1.37	4.75	9	1451	71	43.13	9.7	1.63	412
	Downstream erosional	0.62	1.9	0.76	0.66	1.17	7	685	51	17.09	3.7	0.85	50
	Downstream depositional	0.84	17.5	**23.27**	3.61	4.13	24	1960	130	35.71	27.3	2.03	**1343**
	Independent control	0.68	2.3	1.72	1.16	1.60	5	410	31	23.19	4.4	0.92	204
O River Greta	Upstream Control	0.68	10.2	0.73	0.91	1.89	14	301	103	8.97	6.6	0.78	198
	Downstream	1.49	22.2	2.14	1.49	1.87	8	412	76	19.90	28.7	1.49	**570**
	Downstream erosional	0.84	15.6	0.90	0.84	2.49	13	305	39	17.72	8.4	0.90	348
	Downstream depositional	0.54	8.8	0.54	0.54	1.10	13	212	46	5.15	5.2	0.54	324
	Independent control	1.58	12.0	1.58	1.58	3.81	13	922	128	19.24	8.9	1.58	221
P Arkle Beck	Upstream Control	1.93	6.4	5.96	1.93	6.94	51	1472	133	171.73	**120.9**	2.60	**1251**
	Downstream	0.95	1.1	0.95	1.11	1.29	5	1074	76	15.40	7.1	1.25	117
	Downstream erosional	0.65	0.7	0.65	0.65	1.21	3	643	46	17.03	30.8	1.06	116
	Downstream depositional	0.77	1.5	1.47	1.36	2.00	6	1117	107	24.03	**79.6**	1.57	218
	Independent control	0.92	0.9	1.12	0.92	1.59	4	666	60	25.08	32.4	1.12	132
Q Eggleston Burn	Upstream Control	0.41	0.6	0.41	0.41	0.44	13	1196	46	2.27	2.7	0.41	196
	Downstream	0.80	1.1	0.80	0.80	0.80	12	440	37	3.71	**123.7**	0.80	**511**
	Downstream erosional	0.64	1.0	0.64	0.64	0.64	10	270	45	1.48	**50.8**	0.64	398
	Downstream depositional	0.63	1.0	0.63	0.63	0.63	11	371	68	1.60	**114.6**	0.63	464
	Independent control	1.84	1.8	1.84	1.84	1.84	7	330	83	1.95	2.4	1.84	192
R Hudeshope Beck	Upstream Control	2.53	2.5	2.53	2.53	7.91	11	1396	98	204.97	27.3	2.53	**509**
	Downstream	0.84	0.8	0.84	0.84	4.05	13	537	62	48.14	**124.3**	0.84	**533**
	Downstream erosional	1.02	1.0	1.02	1.02	3.33	11	475	58	64.92	**80.5**	1.02	436
	Downstream depositional	0.51	0.5	0.51	0.51	0.51	15	309	80	9.19	**63.2**	0.51	378
	Independent control	1.07	1.1	1.07	1.07	1.07	13	381	65	15.41	1.1	1.07	453

Catchment	Site	Ag	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	V	Zn
S Bedburn Beck	Upstream Control	0.79	0.8	0.97	0.85	0.85	13	397	202	3.07	4.1	0.79	381
	Downstream	0.75	0.8	0.89	0.75	0.81	11	361	100	2.78	17.7	0.84	454
	Downstream erosional	0.88	0.9	0.94	1.08	0.94	10	548	127	8.44	25.4	0.88	405
	Downstream depositional	1.14	1.1	1.14	1.23	1.57	10	1177	142	35.84	31.8	1.23	467
	Independent control	0.56	0.6	0.56	0.64	0.74	13	219	119	1.25	1.8	0.64	135
T River East Allen	Upstream Control	0.26	**408.0**	0.59	3.62	1.70	70	2994	112	8.33	8.0	1.19	61
	Downstream	0.32	**493.4**	0.58	2.65	2.10	77	4239	104	13.82	8.3	1.40	66
	Downstream erosional	0.44	**480.6**	0.80	3.88	3.93	85	4517	162	19.84	12.8	3.07	104
	Downstream depositional	0.42	**360.3**	0.85	6.84	4.33	82	3157	187	22.63	6.8	3.29	142
	Independent control	0.46	**518.9**	1.39	10.91	5.68	97	3783	281	23.85	10.8	4.12	188

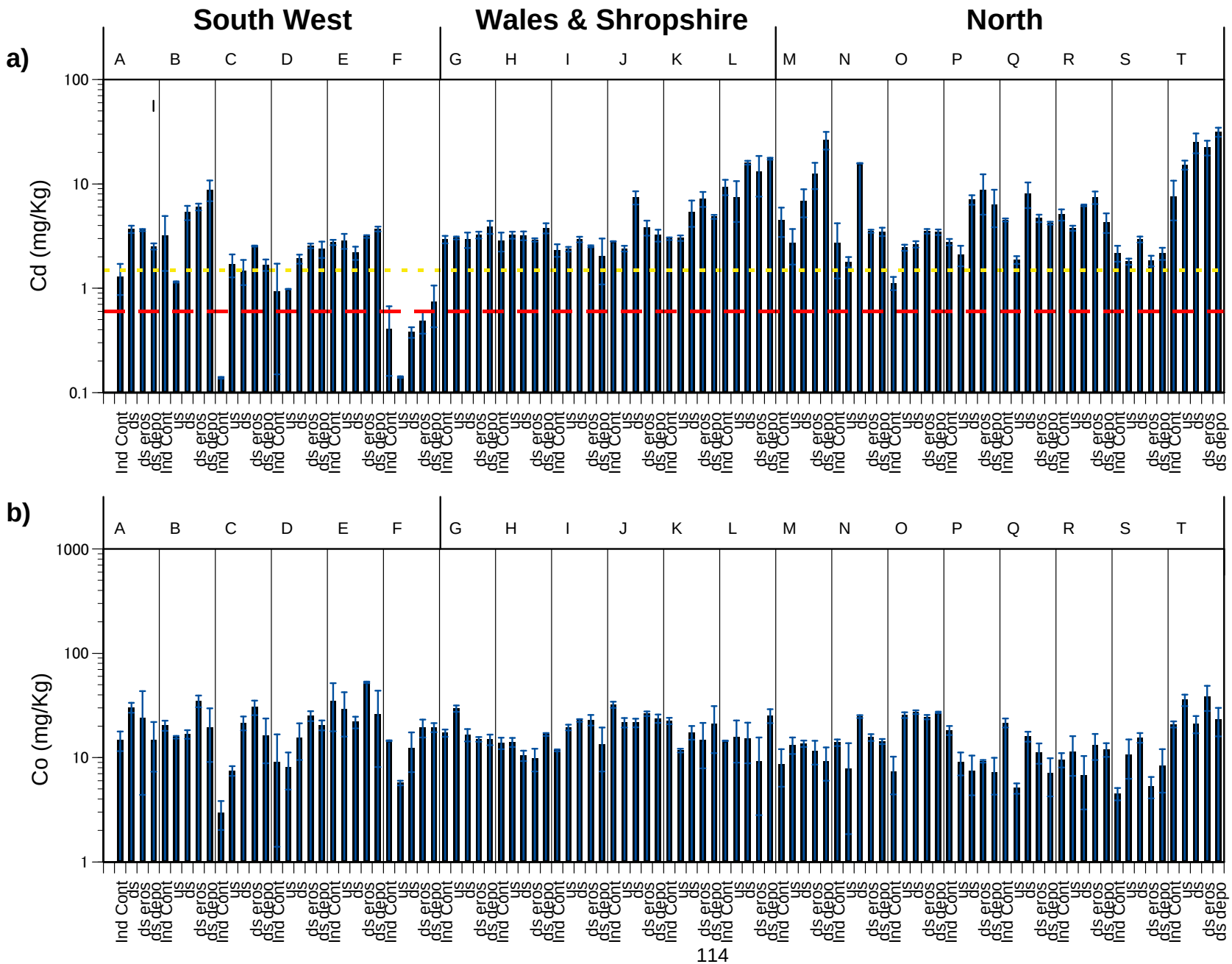
4.3.2 Sediment Geochemistry

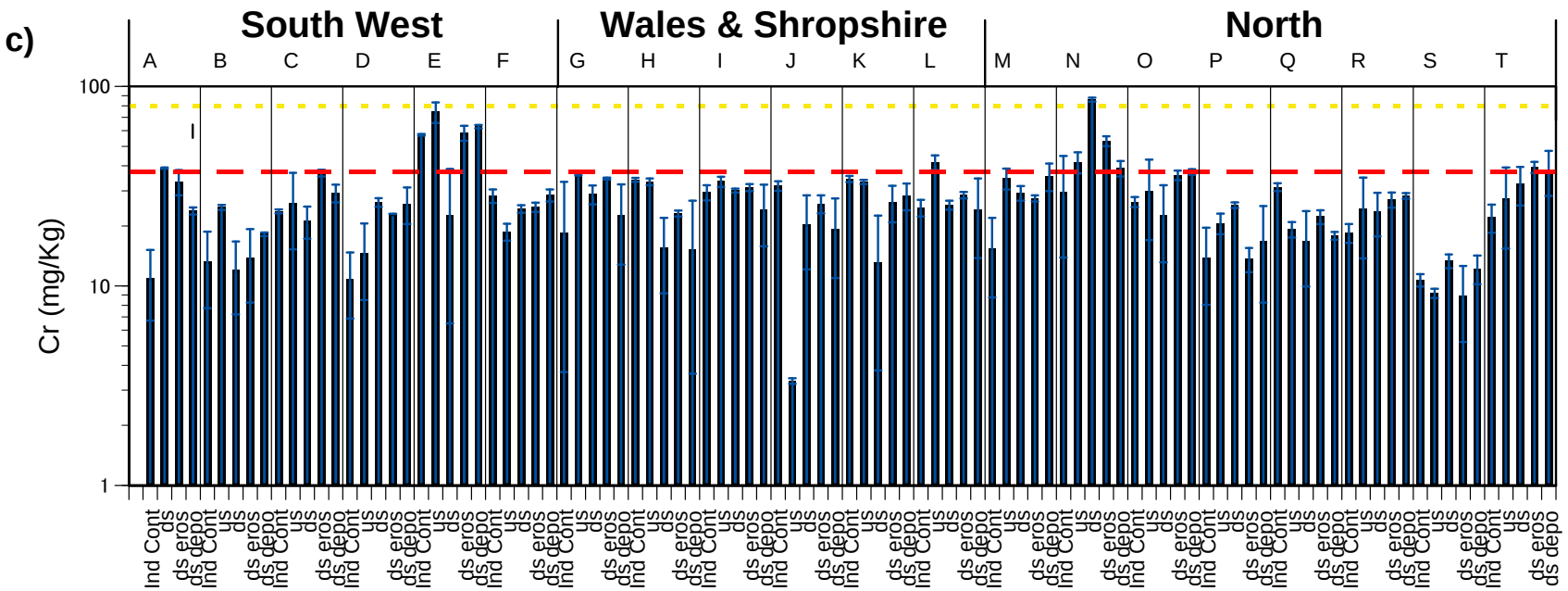
There were clear regional differences in the metal content of river sediments, with higher copper and tin concentrations in the southwest, and higher cadmium, lead and zinc concentrations in Wales and the north. These differences reflect the regional differences in geology. Within regions there were differences among catchments, reflecting the spatially clustered pattern of sites.

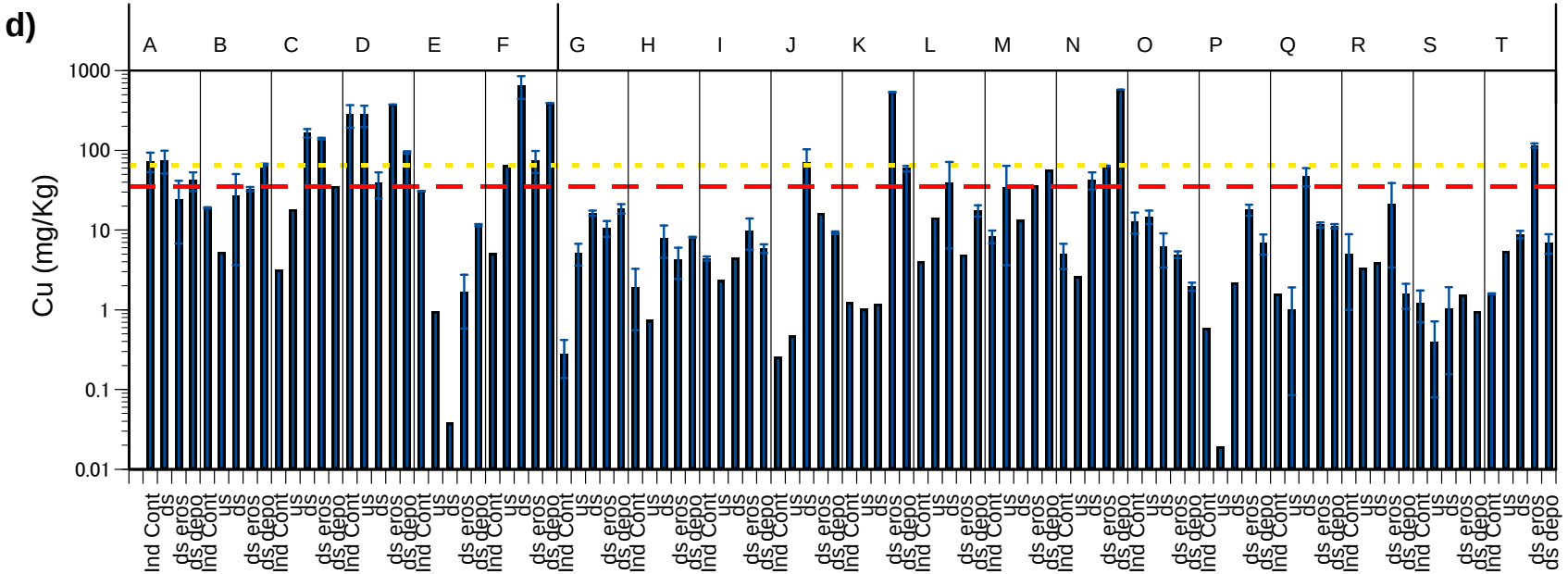
The field sampling strategy appeared to have worked: metal concentrations were typically higher in the sites downstream of the target mining facilities than in the upstream and independent control sites.

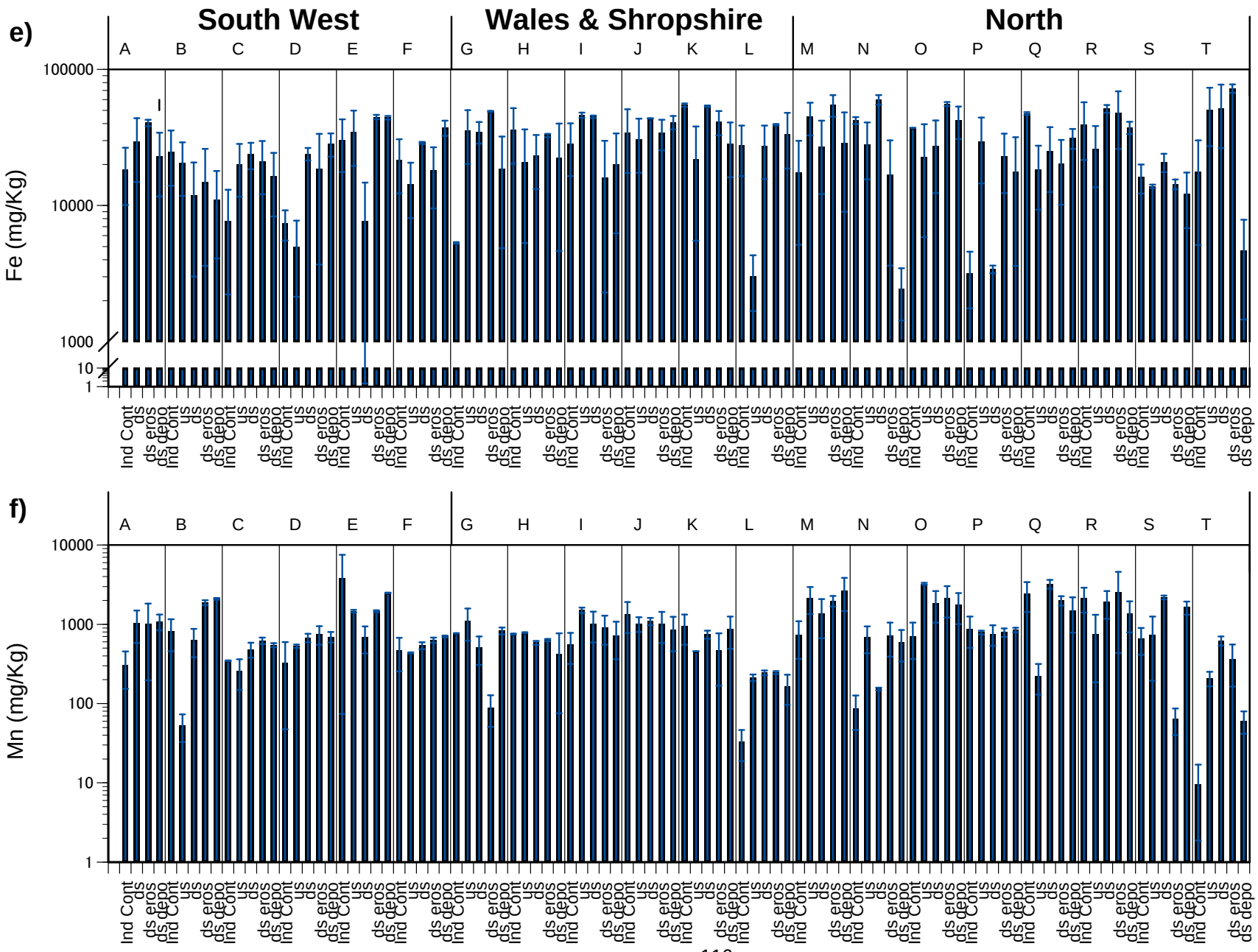
Regarding existing sediment quality guidelines, sites were sampled where the sediment concentrations of copper, chromium, lead, nickel and zinc ranged either side of existing sediment quality guidelines, indicating that, as expected, the sediment at the targeted mine-impacted sites was likely to be causing ecological effects. However, the sediment concentrations of cadmium were in excess of existing sediment quality guidelines at the majority of sites sampled. This suggests either that cadmium is a widespread issue within these mine-impacted sites or that the sediment quality guidelines for cadmium may be overly precautionary.

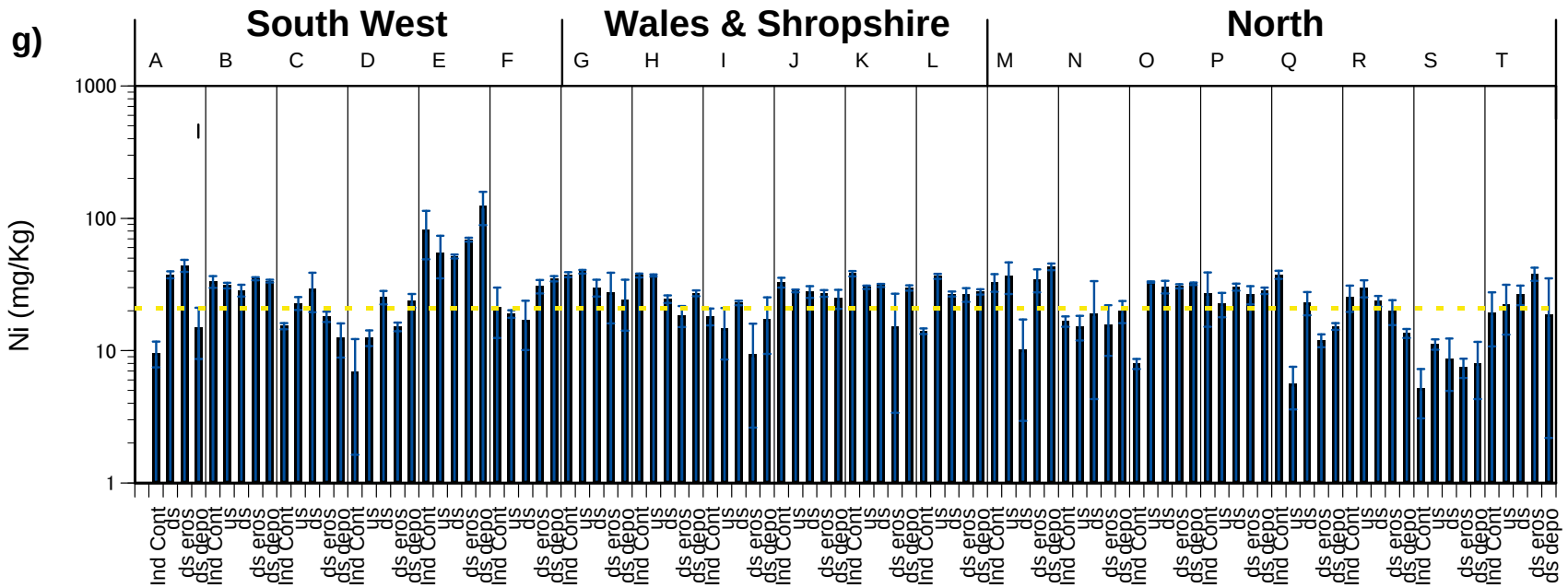
Figure 4.3 Mean (±SE) concentrations (mg Kg^{-1}) of major and trace metals in the river bed sediment collected from mining impacted catchments, together with the existing sediment quality guidelines (CCME = red dashed line; ANZECC and ARMCANZ = gold dotted line) where they exist. a) Cadmium, b) Cobalt, c) Chromium, d) Copper, e) Iron, f) Manganese, g) Nickel, h) Lead, i) Tin and j) Zinc.

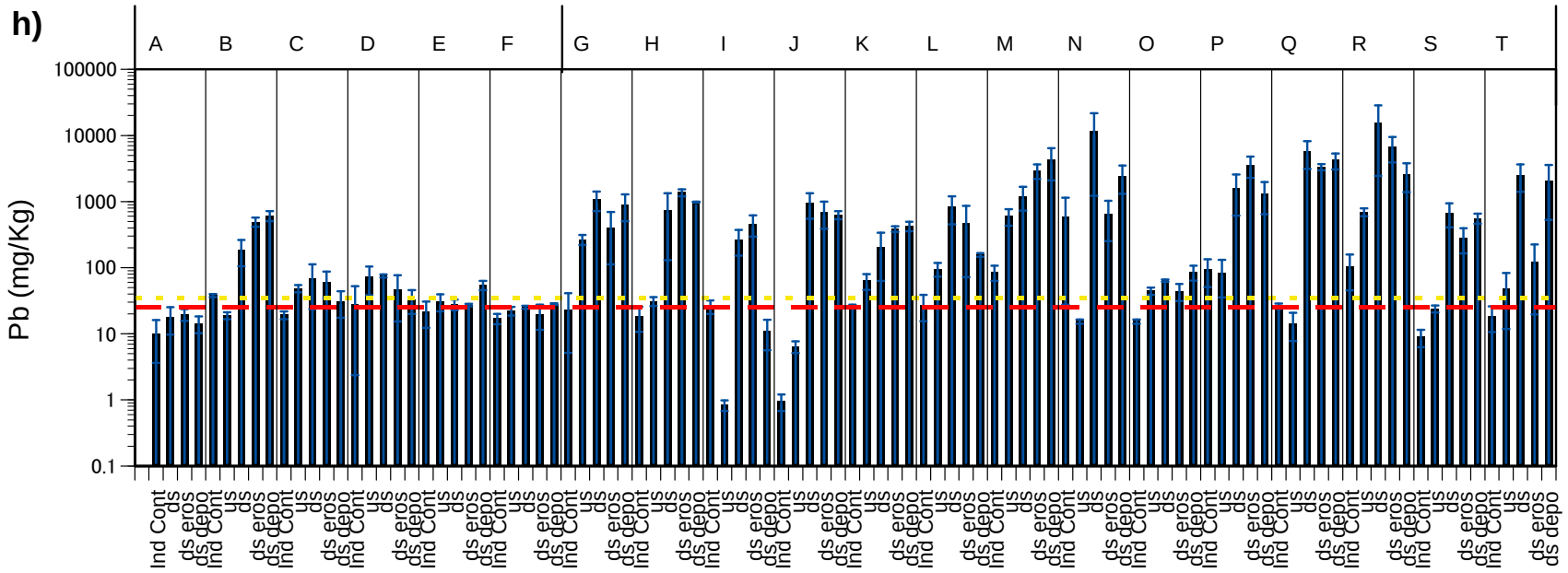

South West
Wales & Shropshire
North
a)
A B C D E F G H I J K L M N O P Q R S T
Cd (mg/Kg)
100
10
1
0.1
b)
Co (mg/Kg)
1000
100
10
1
Ind Cont
ds
ds eros
ds depo
us

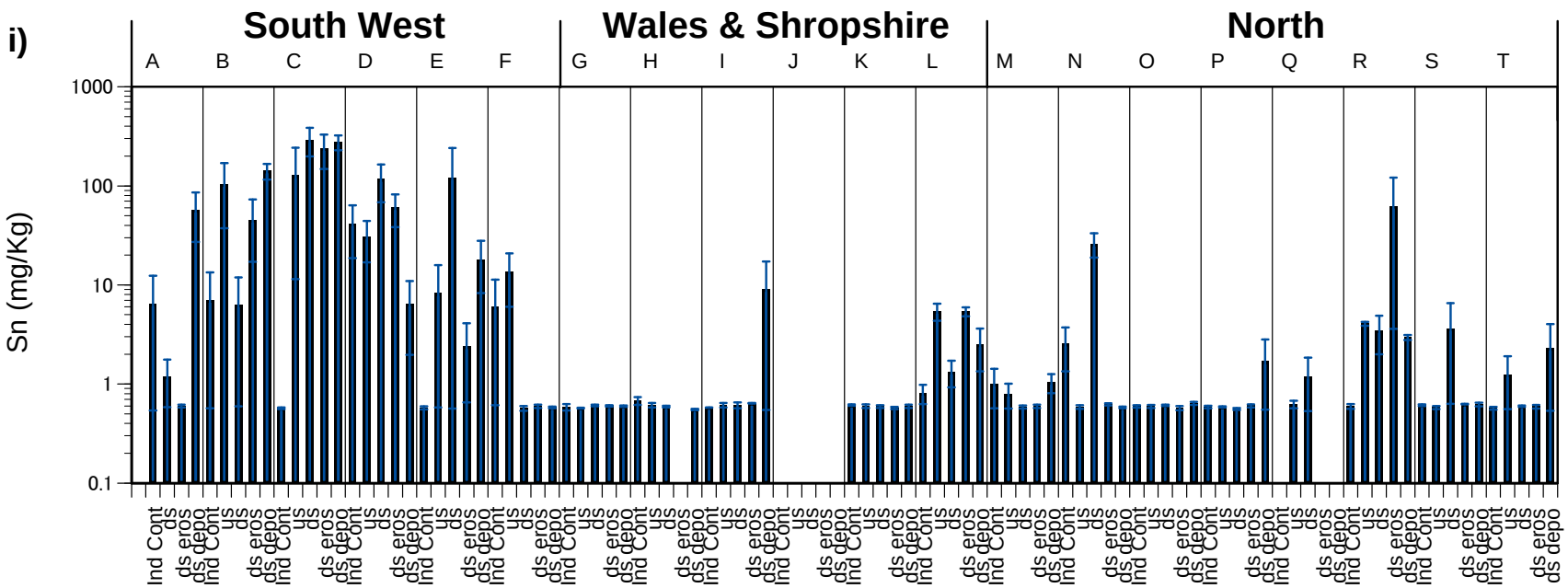

c)
South West
Wales & Shropshire
North
A B C D E F G H I J K L M N O P Q R S T
Cr (mg/Kg)
100
10
1
Ind Cont
ds
ds eros
ds depo
us


d)
A B C D E F G H I J K L M N O P Q R S T
Cu (mg/Kg)
1000
100
10
1
0.1
0.01
Ind Cont
ds
ds eros
ds depo
us


e)
South West
Wales & Shropshire
North
A B C D E F G H I J K L M N O P Q R S T
Fe (mg/Kg)
100000
10000
1000
10
1
Ind Cont
ds
ds eros
ds depo
us
f)
Mn (mg/Kg)
10000
1000
100
10
1


g)
South West
Wales & Shropshire
North
A B C D E F G H I J K L M N O P Q R S T
Ni (mg/Kg)
1000
100
10
1
Ind Cont
ds
ds eros
ds depo
us


h)
A B C D E F G H I J K L M N O P Q R S T
Pb (mg/Kg)
100000
10000
1000
100
10
1
0.1
Ind Cont
ds
ds eros
ds depo
us


i)
South West
Wales & Shropshire
North
A B C D E F G H I J K L M N O P Q R S T
Sn (mg/Kg)
1000
100
10
1
0.1
Ind Cont
ds
ds eros
ds depo
us

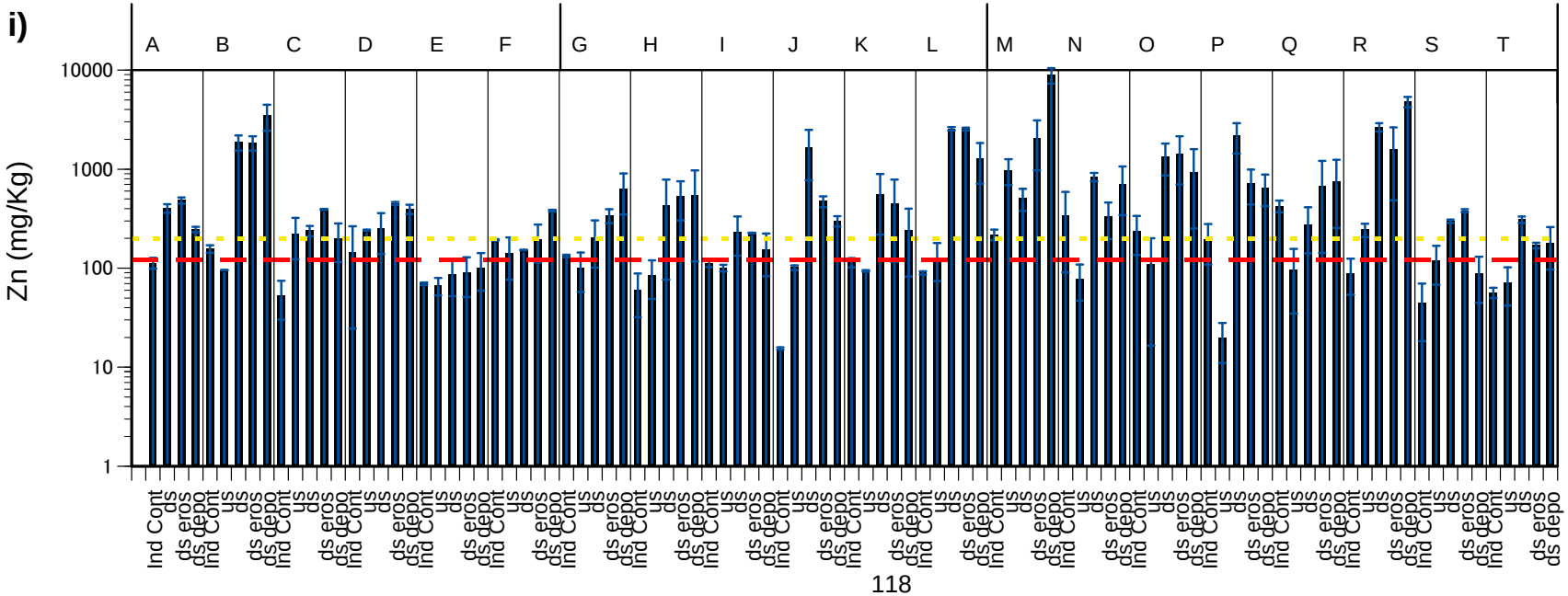
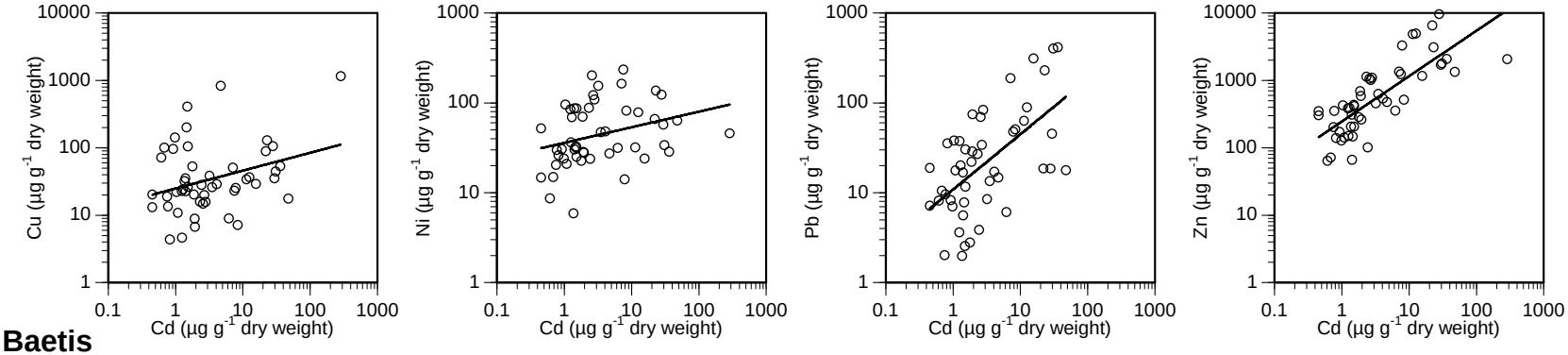
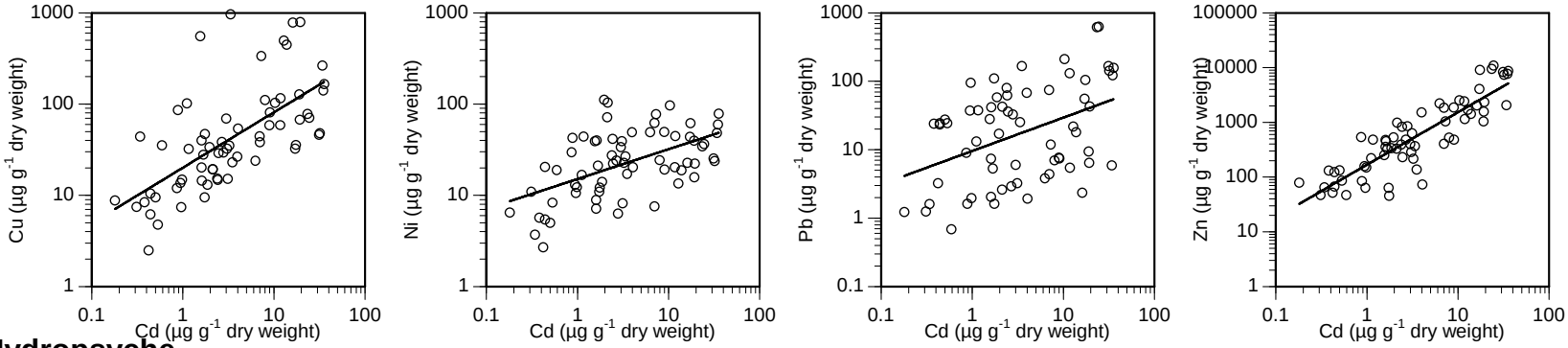

i)
A B C D E F G H I J K L M N O P Q R S T
Zn (mg/Kg)
10000
1000
100
10
1
Ind Cont
ds
ds eros
ds depo
us

Figure 4.4 Relationships between the body burden of cadmium and the body burden of copper, nickel, lead, and zinc within different biomonitor species, a) *Rhyacophila*, b) *Baetis* and c) *Hydropsyche*

a) Rhyacophila



b) Baetis



c) Hydropsyche

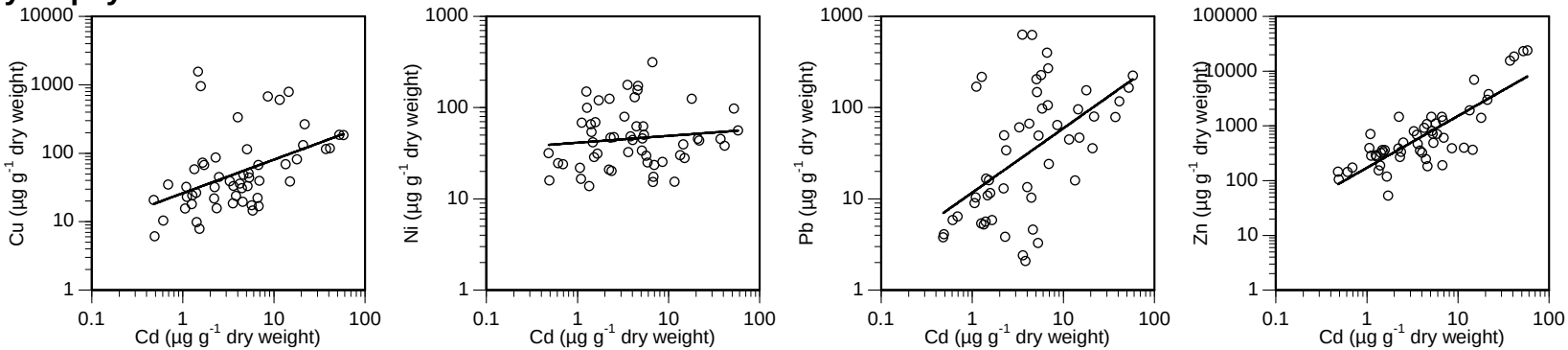
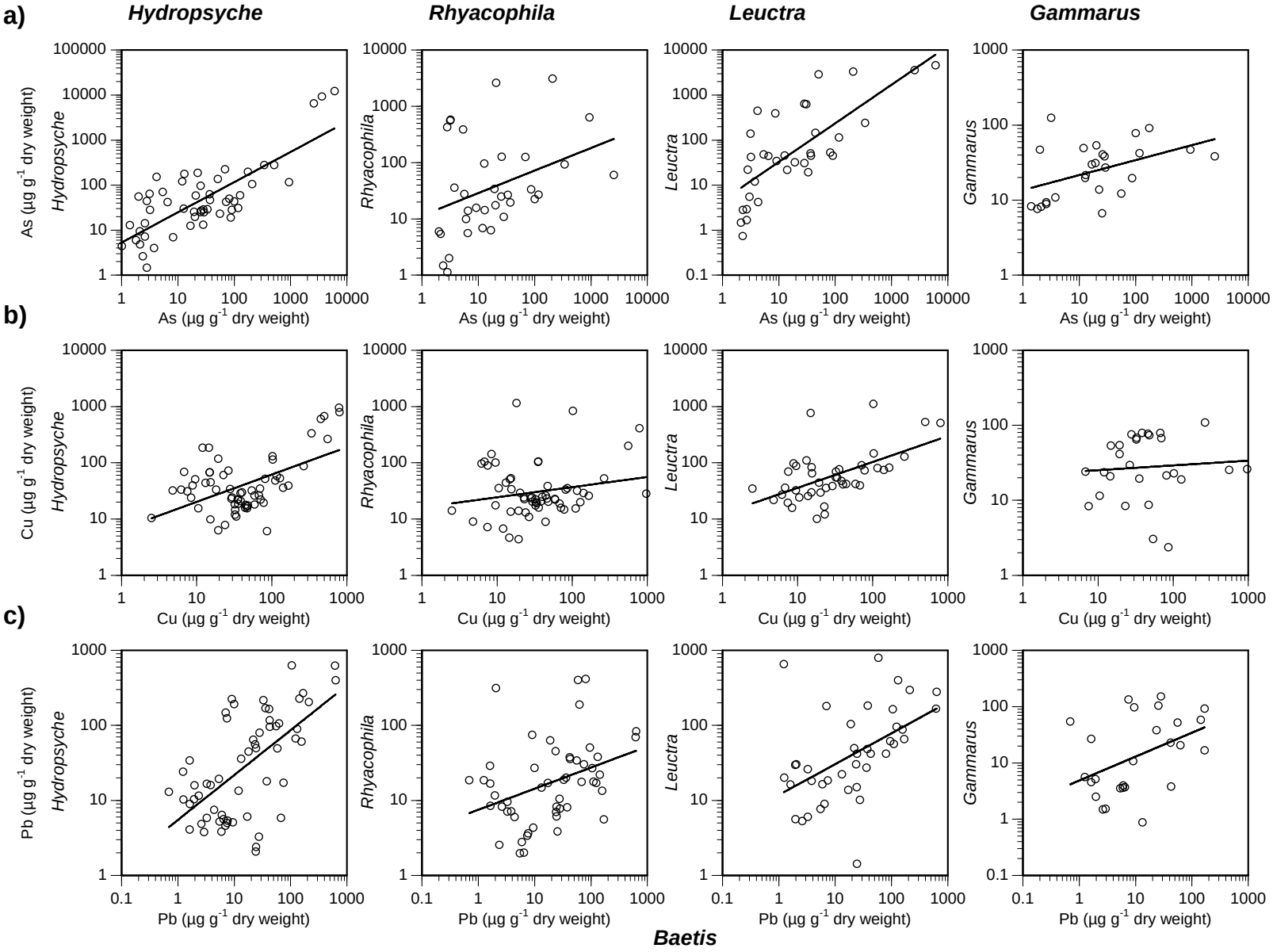


Figure 4.5 Relationships between the body burden of metals in Baetis with the corresponding metal in different biomonitor species, a) arsenic, b) copper and c) lead.



4.3.3 Relationships between biomonitor body burden and sediment metal concentrations

The aim of the biomonitor analysis was to provide a measure of local bioavailability of metals at sites contaminated by mining, that could then be used to understand the ecological responses to sediment contaminated by mining, either directly within or in contact with the sediment or via sediment exchange with the water column. Using the data on biomonitor body burden and the sediment metal concentrations, we addressed **Objective 4a** [**Establish the sensitivity of benthic invertebrate species to metal bioavailability (using partial ordination techniques) and, on that basis, devise a new diagnostic index that can be used to determine failure of test sites as a consequence of contaminated sediments**.].

a Relationships among metals within species

Within individual species there were strong correlations between the body burdens of various metals (Figure 4.4). In simple terms, individuals which had a high body burden of, for example, cadmium tended to also have a high body burden of other metals (e.g. copper, lead and zinc). These results potentially reflect,

a) the co-occurrence of certain metals due to geology,
b) variation in bioavailability due to local conditions, affecting all metals present at a site,
c) behaviour of metals, particularly with regards physiology (e.g. cadmium can act as a surrogate for zinc).

These results can be used to establish which metals present as body burden in the biomonitor taxa best reflect local metal bioavailability across species.

b Relationships among species

Due to variation in species occurrence across the sites surveyed, it was decided to collect multiple biomonitor species from each site for analysis of body burden. The objective was to establish relationships between species so that the bioavailability could be predicted, using those species that were present, at sites where the favoured biomonitor species was absent. Strong correlations were generally apparent between the body burden of metals in *Baetis* (the most frequently encountered species) and the other species collected as biomonitors, particularly *Hydropsyche* and *Leuctra* (Figure 4.5). It was noted that there was a poor relationship between the body burden of copper in *Baetis* and that in *Gammarus*, possibly due to physiological differences: haemocyanin, the respiratory protein in Crustacea contains copper. Nevertheless, *Gammarus* was encountered at the least number of sites.

c Relationships with sediment chemistry

Whilst local conditions may influence the bioavailability of metals, the body burden of metals in the biomonitor species was expected to be correlated with the metal concentrations in the sediment to a degree. The extent to which the body burden and sediment concentrations are correlated will depend on the bioavailability of the metal, and the physiology of the organism.

Copper and lead showed the strongest relationships between body burden and sediment concentrations (Figure 4.6b & d), significant for all four species tested *Baetis, Hydropsyche, Leuctra* and *Rhyacophila*. The residuals of the relationships between sediment concentrations and body burdens of copper and lead in all four taxa (with the exception of copper in *Rhyacophila*) were significantly negatively correlated with the pH measured at the time of sampling, indicating an influence of pH (or a correlated variable) on bioavailability. For copper, the residuals were also correlated positively with conductivity for all four taxa, although (with the exception of *Rhyacophila*) not as strongly as pH.

Due to the low frequency of occurrence *Gammarus* was not used for this analysis. Furthermore, preliminary analysis indicated that the relationship between copper in the sediment and copper in *Gammarus* was weak, which again may be related to haemocyanin, the respiratory protein in Crustacea which contains copper.

The relationship between cadmium concentration in the sediment and the body burden of cadmium was not significant for any taxon (Figure 4.6a).

The relationship between nickel in the sediment and the body burden of nickel was significant for *Leuctra* and *Rhyacophila* (Figure 4.6c). Again, pH was negatively correlated with the residuals of the relationship.

The relationship between the concentration of zinc in the sediment and the body burden of zinc was significant for *Baetis*, *Hydropsyche* and *Leuctra* (Figure 4.6e). For Baetis the residuals were significantly positively correlated with conductivity, for *Leuctra* and *Rhyacophila* negatively with pH.

Figure 4.6a Relationships between the concentrations of cadmium in fine sediments of the stream bed from where the animals were collected and cadmium in the tissues of *Baetis, Hydropsyche*, *Leuctra and Rhyacophila.* Also shown is the ability of pH and conductivity to explain the residuals of the relationship for each species. Lines shown where significant.

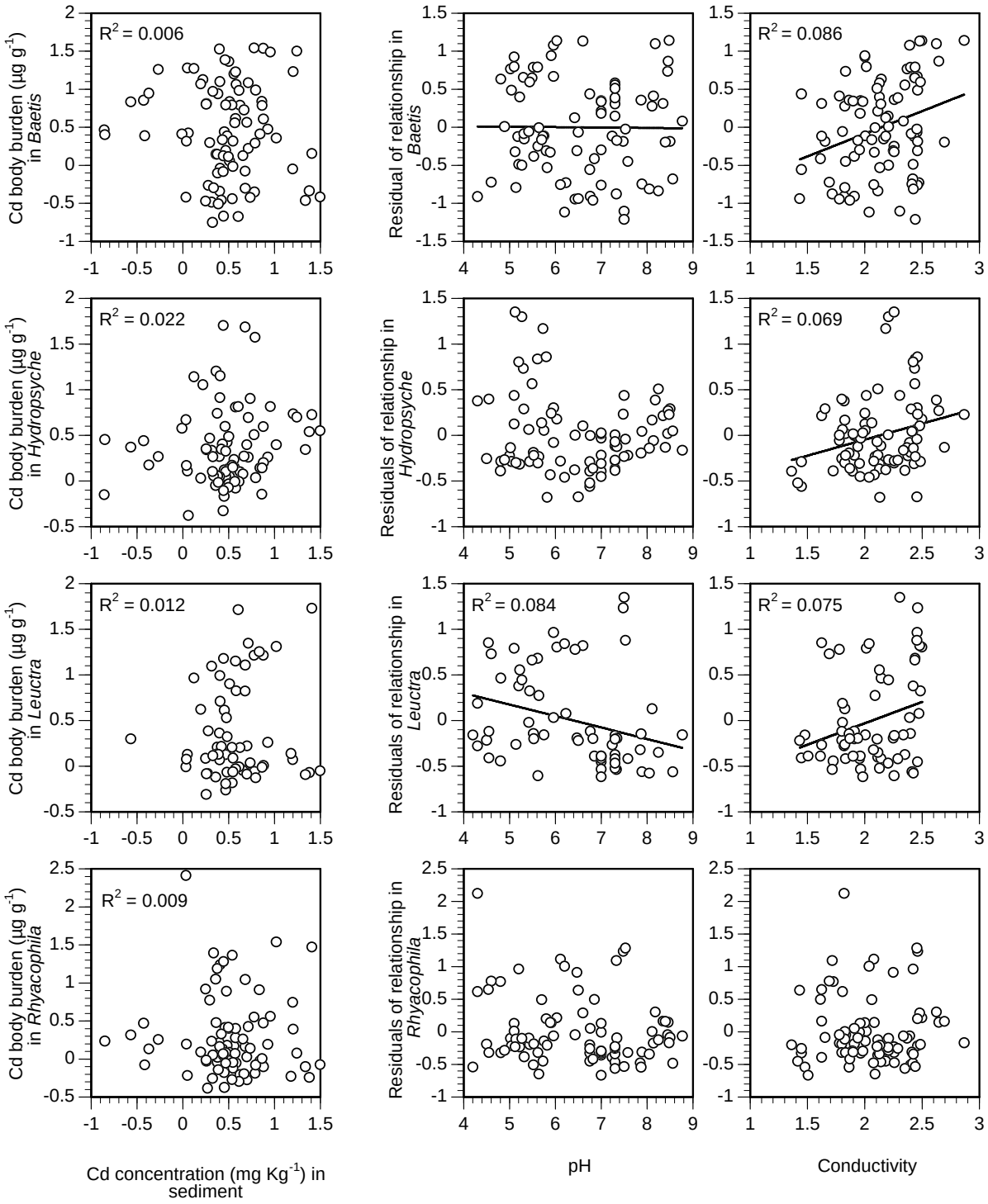


Figure 4.6b Relationships between the concentrations of copper in fine sediments of the stream bed from where the animals were collected and copper in the tissues of *Baetis, Hydropsyche*, *Leuctra and Rhyacophila.* Also shown is the ability of pH and conductivity to explain the residuals of the relationship for each species. Lines shown where significant.

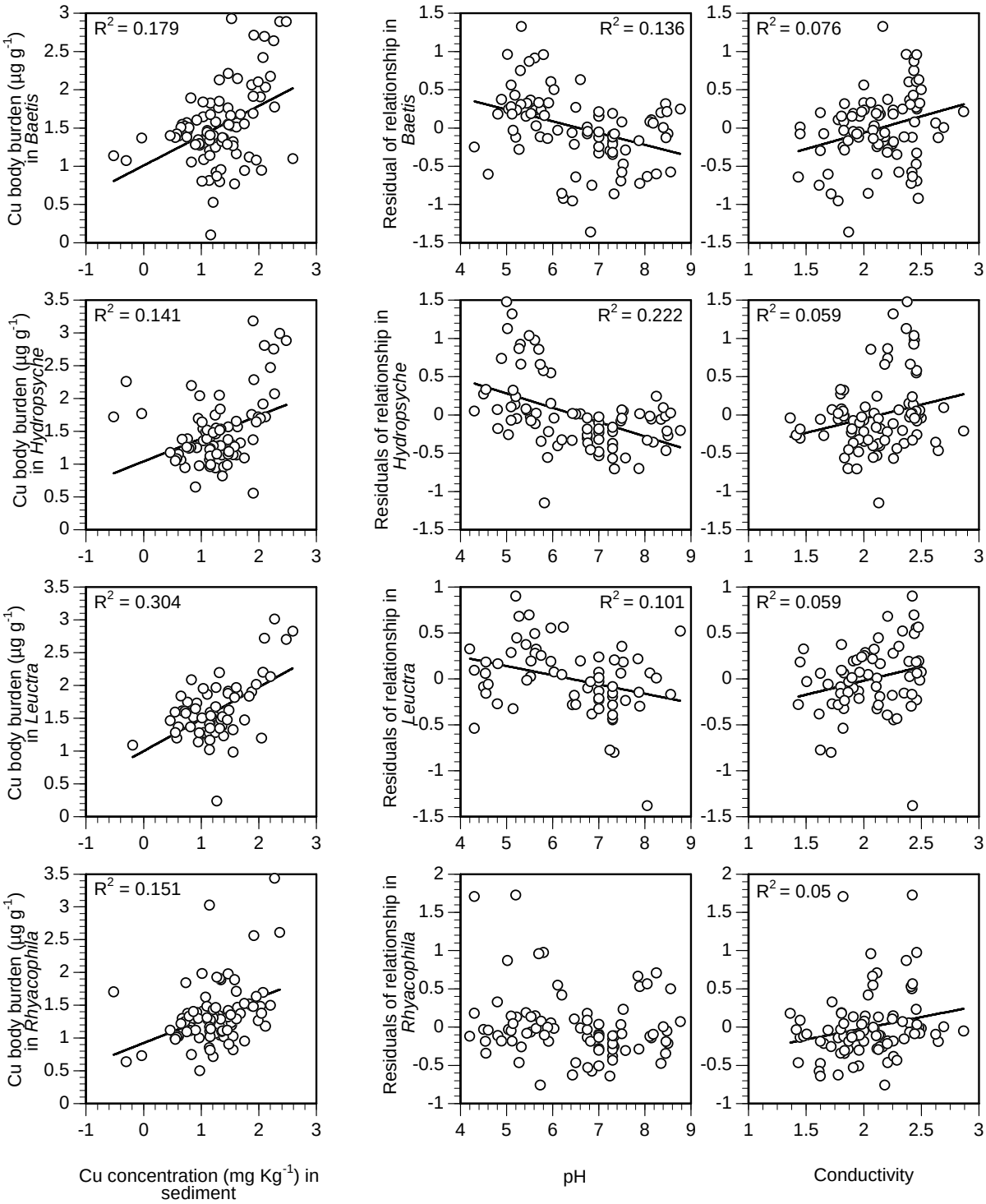


Figure 4.6c Relationships between the concentrations of nickel in fine sediments of the stream bed from where the animals were collected and nickel in tissues of *Baetis, Hydropsyche*, *Leuctra and Rhyacophila.* Also shown is the ability of pH and conductivity to explain the residuals of the relationship for each species. Lines shown where significant.

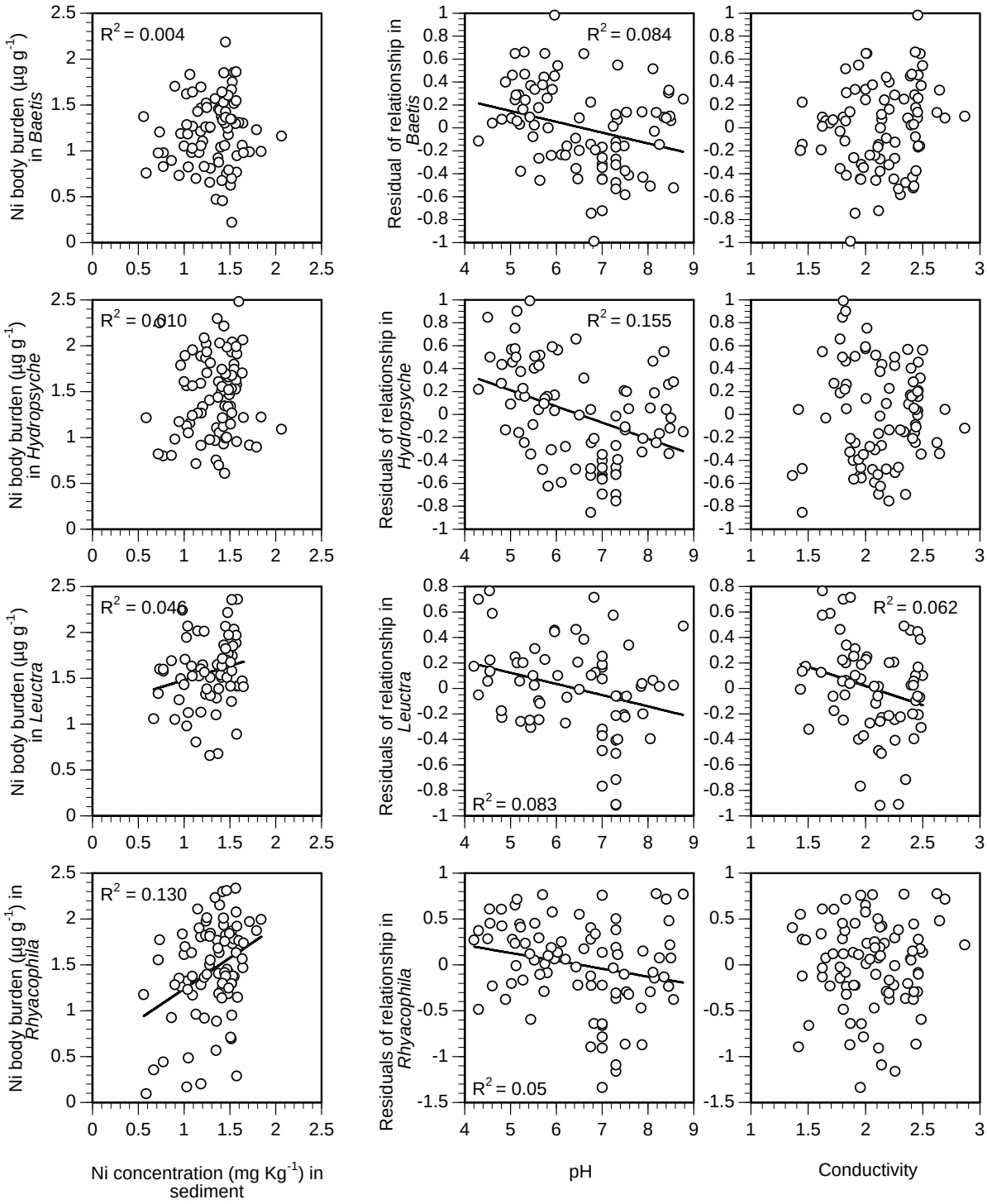


Figure 4.6d Relationships between the concentrations of lead in fine sediments of the stream bed from where the animals were collected and lead in tissues of *Baetis, Hydropsyche*, *Leuctra and Rhyacophila.* Also shown is the ability of pH and conductivity to explain the residuals of the relationship for each species. Lines shown where significant.

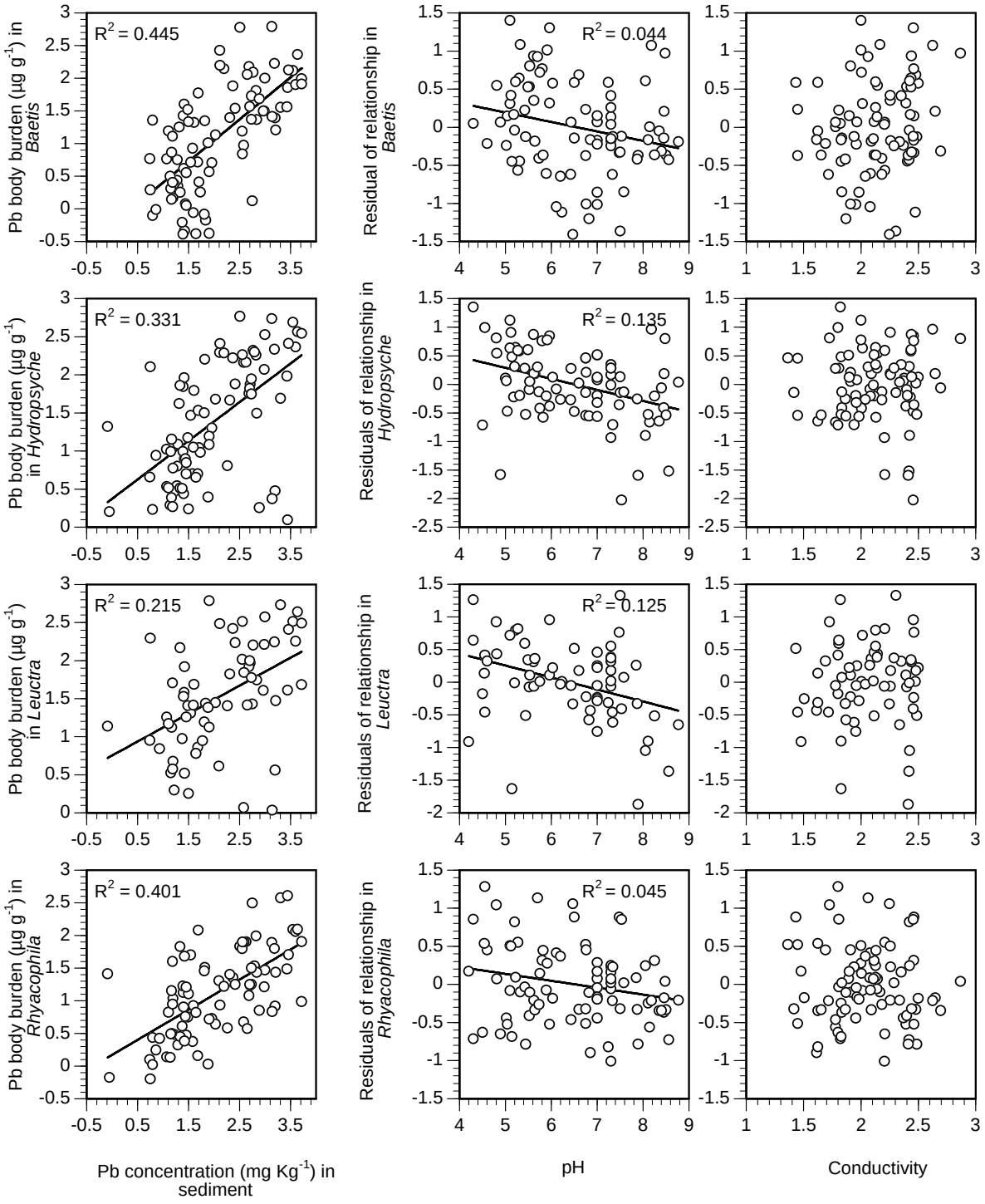
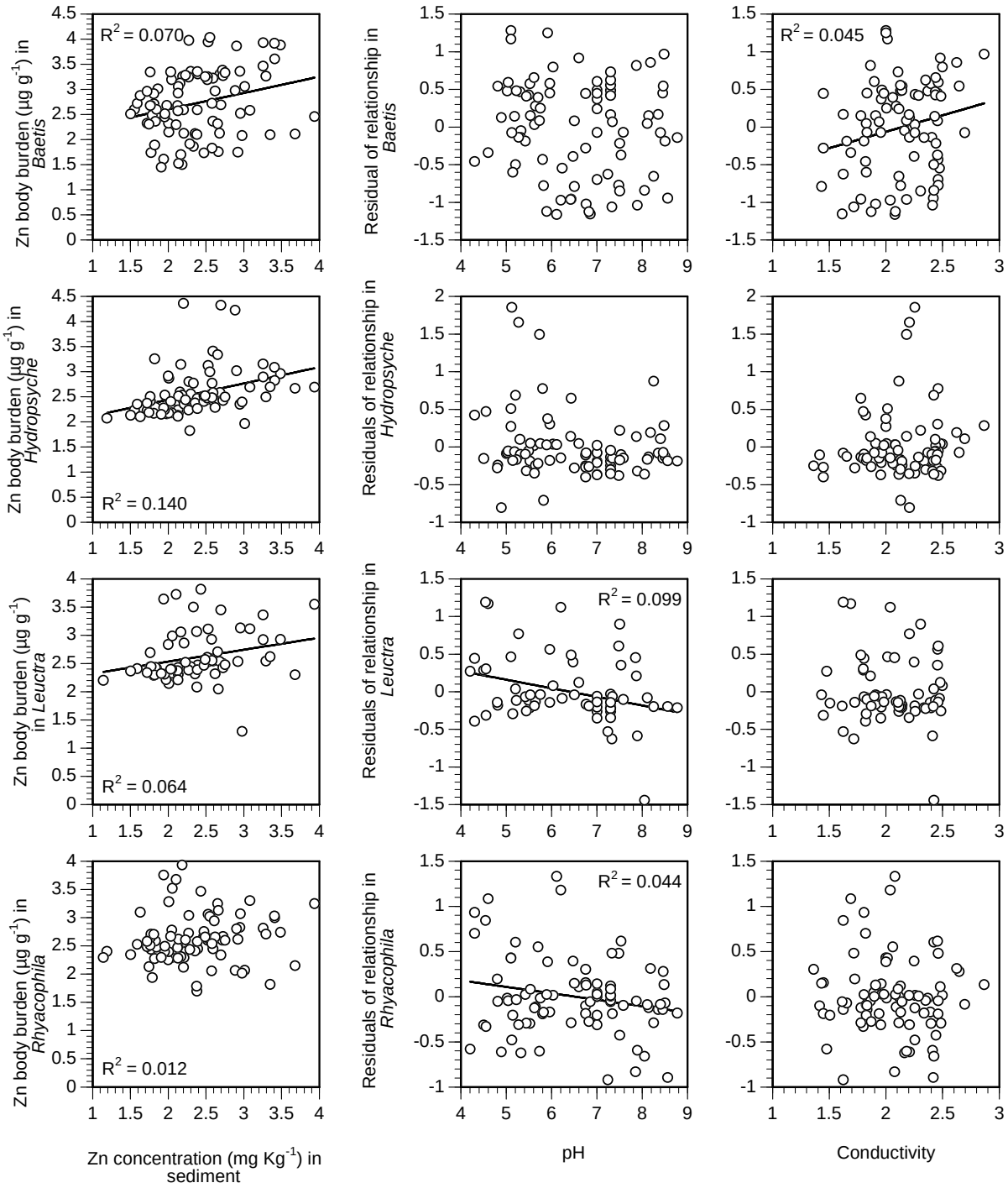


Figure 4.6e Relationships between the concentrations of zinc in fine sediments of the stream bed from where the animals were collected and zinc in tissues of *Baetis, Hydropsyche*, *Leuctra and Rhyacophila.* Also shown is the ability of pH and conductivity to explain the residuals of the relationship for each species. Lines shown where significant.



d Influence of environmental variables on relationship between body burden and sediment chemistry

The significant relationships between the sediment-body burden residuals and environmental variables indicates an influence of environmental conditions on bioavailability. Hence, a multiple regression approach was used to identify the environmental variables which were best related to the body burden of metals. A stepwise selection procedure was used in general linear models in SAS. Variables are entered into the model in sequence according to their additional explanatory power. Variables can also be removed if subsequent combinations of variables offer more explanatory power. F-tests and AIC rules are used to determine inclusion and the stopping point. Various descriptors of the sediment (metal concentration in sediment (mg kg^{-1}), reach scale average mass of fine sediment, reach scale average mass of metal (mg m^{-2}), organic content of fine sediment, sediment phi score), water (pH, conductivity) and site characteristics (width, average depth, distance from source, altitude, river slope) were offered as explanatory variables for determining the body burden of arsenic, cadmium, copper, nickel, lead and zinc. The first entered variable and the variables included in the optimal model were recorded.

Table 4.8 First entered variables and variables included in optimal models to explain body burden of metals in Baetis, Hydropsyche, Leuctra and Rhyacophila. Order of variables included as given and positive (+) or negative (-) influence indicated.

Body burden	Taxon	First variable entered	R^2	Optimal model	R^2
As	*Baetis*	Cu_mgkg	0.301	Cu_mgkg -Pb_mgkg -pH +Cond -Cd_mgkg +Zn_mgkg	0.695
	Hydropsyche	-Pb_mgm	0.123	-Pb_mgm +Cond +Cu_mgkg -pH	0.364
	Leuctra	Cu_mgm	0.295	Cu_mgkg -Pb_mgm +Cond	0.400
	Rhyacophila	-Pb_mgm	0.120	-Pb_mgm +Zn_mgkg +Cu_mgkg	0.291
Cd	*Baetis*	altitude	0.197	-altitude +Pb_mgkg	0.320
	Hydropsyche	conduct	0.088	Cond +depth -Cu_mgm	0.242
	Leuctra	Cu_mgkg	0.154	Cu_mgkg +Ni_mgkg	0.241
	Rhyacophila	Cu_mgkg	0.069	Cu_mgkg -pH	0.109
Cu	*Baetis*	Cu_mgkg	0.169	Cu_mgkg -pH +Cond -Cd_mgkg	0.455
	Hydropsyche	pH	0.164	-pH +Cu_mgkg +Cond -Ni_mgm	0.399
	Leuctra	Cu_mgkg	0.293	Cu_mgkg –pH +Cond -Zn_mgkg	0.484
	Rhyacophila	Cu_mgkg	0.141	Cu_mgkg -pH -Pb_mgkg +Cond +%Organic +depth +Cd_mgkg	0.388
Ni	*Baetis*	-altitude	0.227	-alt	0.227
	Hydropsyche	-pH	0.146	-alt +slope +Cd_mgkg	0.265
	Leuctra	- Ca_mgkg	0.130	-Ca_mgkg	0.130
	Rhyacophila	Ni_mgkg	0.119	Ni_mgkg -Ca_mgkg	0.192
Pb	*Baetis*	Pb_mgkg	0.438	Pb_mgkg -pH	0.530
	Hydropsyche	Pb_mgkg	0.323	Pb_mgkg -pH	0.409
	Leuctra	Pb_mgkg	0.256	Pb_mgkg -pH +substrate -Ni_mgkg	0.460
	Rhyacophila	Pb_mgkg	0.440	Pb_mgkg -pH +altitude +source	0.552
Zn	*Baetis*	Conductivity	0.087	Pb_mgkg +substrate	0.177
	Hydropsyche	Zn_mgkg	0.132	Zn_mgkg	0.132
	Leuctra	Zn_mgkg	0.065	Zn_mgkg -pH	0.178
	Rhyacophila	Ni_mgkg	0.064	Ni_mgkg	0.064

Arsenic body burden was best explained by either a positive influence of copper concentration in the sediment or a negative influence of lead (Table 4.8). Both these variables were included in all optimal models. Arsenic concentration in the sediment was not measured. Cadmium body burden was best explained by copper concentration in the sediment or other variables (Table 4.8). Nickel body burden was best described by a variety of variables, with the exception of *Rhyacophila* where nickel concentration together with a negative influence of calcium concentration gave the optimal model.

Both copper and lead were best described by the concentration of that metal in the sediment in combination with negative influence of pH, with some other variables (Table 4.8). For both copper and lead, the concentration of metal in the sediment and pH were the first two variables included into the models for all taxa (with the exception of copper in *Rhyacophila*), and these two variables alone explained a substantial proportion of the variation in body burden (Table 4.9).

Zinc body burden was best described by zinc concentration in the sediment for *Hydropsyche* and with a negative influence of pH for *Leuctra*.

In almost all cases the concentration of metals in the sediment (metal_mgkg) was a better descriptor of metal body burden than average mass of metals on the river bed (metal_mgm = metal concentration x average fine sediment mass). This may be due to errors from the two measurements being compounded when estimating metal mass m^{-2}, or it could be due to how the biomonitors accessed metals from the sediment. It should be noted that as a separate variable, substrate phi (a measure of whole bed granulometry based on visual estimates, inversely related to particle size) was included in two optimal models (lead in *Leuctra* and zinc in *Baetis*) and % organic of fine sediment in one optimal model (copper in *Rhyacophila*), suggesting that more fine particles in the river bed may influence the uptake of metals.

For arsenic, cadmium and copper, conductivity was frequently included in the optimal model, positively correlated with increased body burden.

Overall these results suggest a strong association between biomonitors and copper and lead in the sediment, and for certain taxa, zinc and nickel. There was also a pronounced negative influence of pH.

Table 4.9 First two variables entered into models to explain body burden of copper and lead in Baetis, Hydropsyche, Leuctra and Rhyacophila. Order of variables included as given and positive (+) or negative (-) influence indicated. R^2, adjusted for number of variables, of two variable model and optimal model given.

	Taxon	2 variable model	R^2	Optimal model R^2
Cu	*Baetis*	Cu_mgkg -pH	0.274	0.455
	Hydropsyche	-pH +Cu_mgkg	0.368	0.399
	Leuctra	Cu_mgkg -pH	0.354	0.484
	Rhyacophila	Cu_logmgkg -Pb_logmgkg	0.193	0.388
Pb	*Baetis*	Pb_mgkg -pH	0.530	0.530
	Hydropsyche	Pb_mgkg -pH	0.423	0.423
	Leuctra	Pb_mgkg -pH	0.391	0.460
	Rhyacophila	Pb_mgkg -pH	0.501	0.552

4.3.4 Relationships between biomonitor body burden and sediment metal concentrations derived from samples used for source apportionment

As part of the tasks comprising work package 3, where sediment source apportionment was undertaken to identify the sources of metals arriving at the point of impact, the well-mixed fraction (< 63 µm) of fine-grained sediment was collected from the most downstream site in the 20 study catchments (see Section 6). Each bed sediment sample (total volume of 5 L) comprised a composite of two sub-samples (~ 2.5 L each) retrieved from different points in the channel at the outlet of each study catchment. A total of 12 (10 in the case of the River Greta) composite samples were retrieved from each catchment outlet (see Table 6.1). These samples were oven-dried at 40 °C, manually disaggregated using a rubber-tipped pestle and homogenised using a 63 µm sieve (Collins et al., 1997). Concentrations of potential geochemical fingerprint properties were determined using a combination of ICP-OES and ICP-MS, following an aqua regia acid digest. For full details see Section 6.

Hence, an independent estimate of the concentration of metals in the sediment, based on a larger sample of fine material, was available for one site in each catchment. Although this information was available from fewer sites, the larger number of replicates and larger volume collected could address any spatial heterogeneity in the composition of fine sediments, which may have confounded relationships between sediment metal concentrations and the body burden of metals in the biomonitor species. Hence, it was decided to determine the relationships between the body burden of metals in the biomonitor species and the concentration of metals in the samples used for source apportionment. Furthermore, these sediment metal concentrations were based on the finer fraction (< 63 µm), which may be more biologically active, and thus provide a better estimate of ecological risk.

As fewer sites were used for source apportionment, fewer significant relationships were identified than in the larger dataset (*cf* Section 4.3.4c and Figure 4.6). Nevertheless, significant ($p \leq 0.05$) or close to significant ($p \leq 0.1$) relationships were found between the concentrations of lead, copper and arsenic in the fine fraction of sediment and the tissue concentrations in *Baetis*, *Hydropsyche* and *Rhyacophila* (Figure 4.7). There was also a significant relationship between cadmium concentration in the fine fraction of sediment and in the tissues of *Hydropsyche*, and a close to significant relationship for zinc concentrations in *Hydropsyche*. Of the metals of less interest, there was a relationship between the concentrations of cobalt in the fine fraction of sediment (< 63 µm) and in the tissue concentrations of *Leuctra* and close to significant for *Baetis*. Similarly, there was a close to significant relationship for manganese in the sediment and in the tissues of *Leuctra*. For the other metal – biomonitor combinations, particularly nickel and zinc, patterns were apparent, but a low number and narrow range of data points resulted in non-significant relationships.

No relationships were seen between chromium concentration in the sediment and that in the tissues of the biomonitor taxa, which suggests that factors other than total concentration influence the biological availability of chromium in these sites.

As well as supporting the data from the wider survey of biomonitor taxa, particularly regarding copper and lead, these data indicate that arsenic and to an extent cadmium bioavailability can be predicted from sediment concentrations. The lack of a more substantial result with cadmium is probably due to the difficulties of quantifying this metal, which is frequently found at concentrations close to the detection limit.

Figure 4.7a Relationships between the concentrations of metals and metalloids (As, Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn) in fine sediments (< 63 µm) collected for source apportionment and corresponding concentrations in the tissues of *Baetis*. Solid lines shown where significant: dashed lines close to significant.

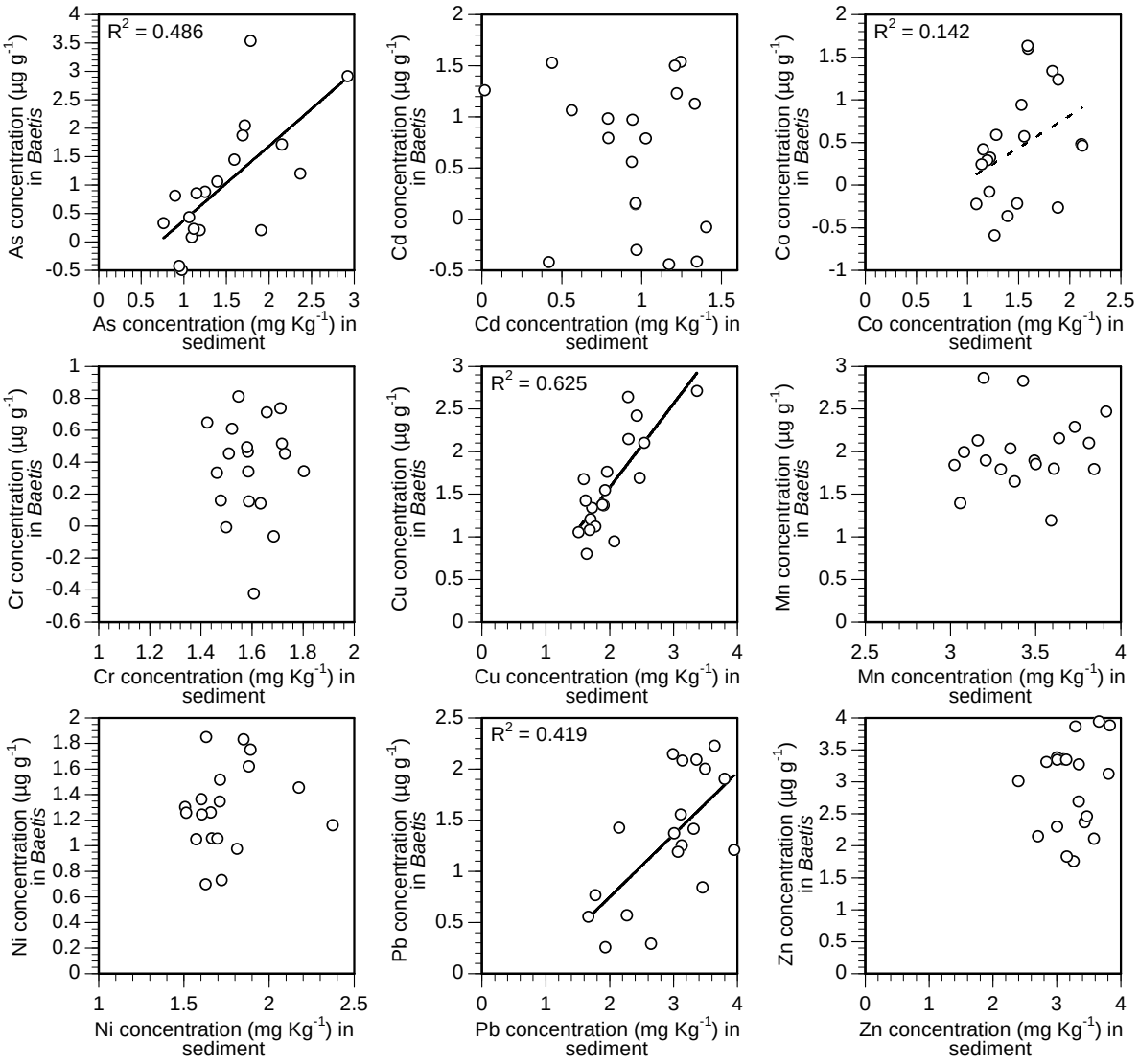


Figure 4.7b Relationships between the concentrations of metals and metalloids (As, Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn) in fine sediments (< 63 µm) collected for source apportionment and corresponding concentrations in the tissues of *Hydropsyche*. Solid lines shown where significant: dashed lines close to significant.

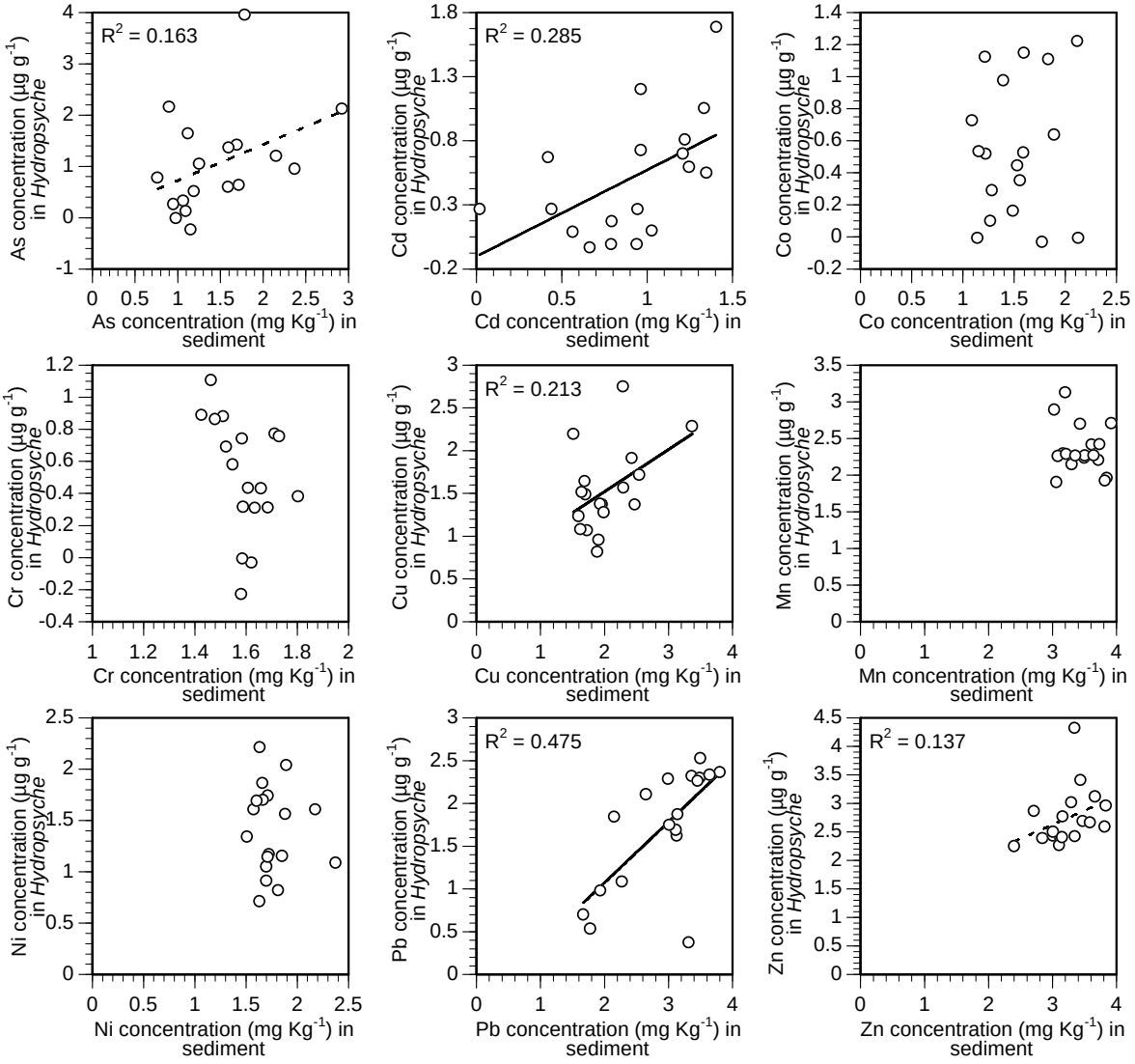


Figure 4.7c Relationships between the concentrations of metals and metalloids (As, Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn) in fine sediments (< 63 µm) collected for source apportionment and corresponding concentrations in the tissues of *Leuctra*. Solid lines shown where significant: dashed lines close to significant.

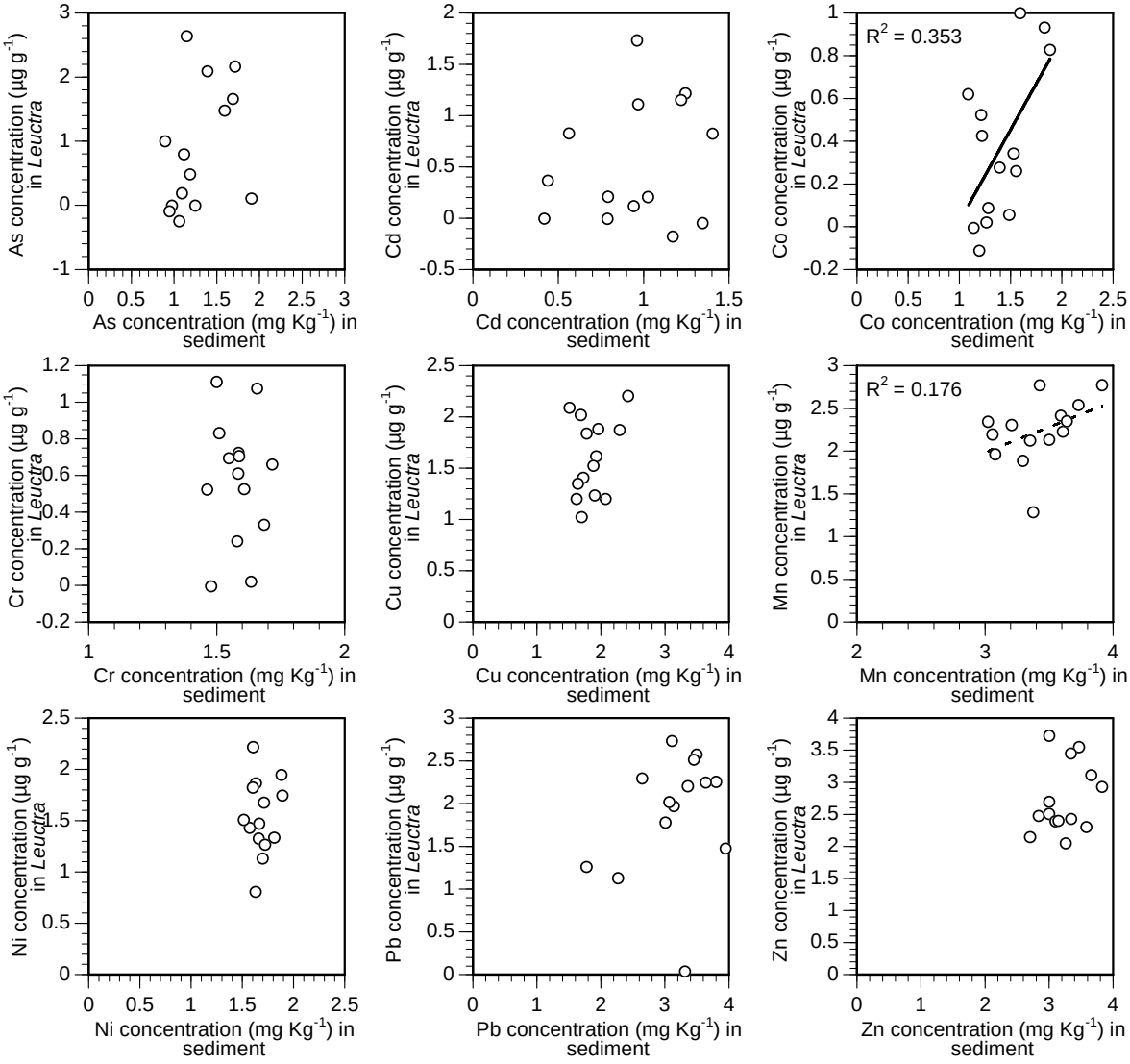
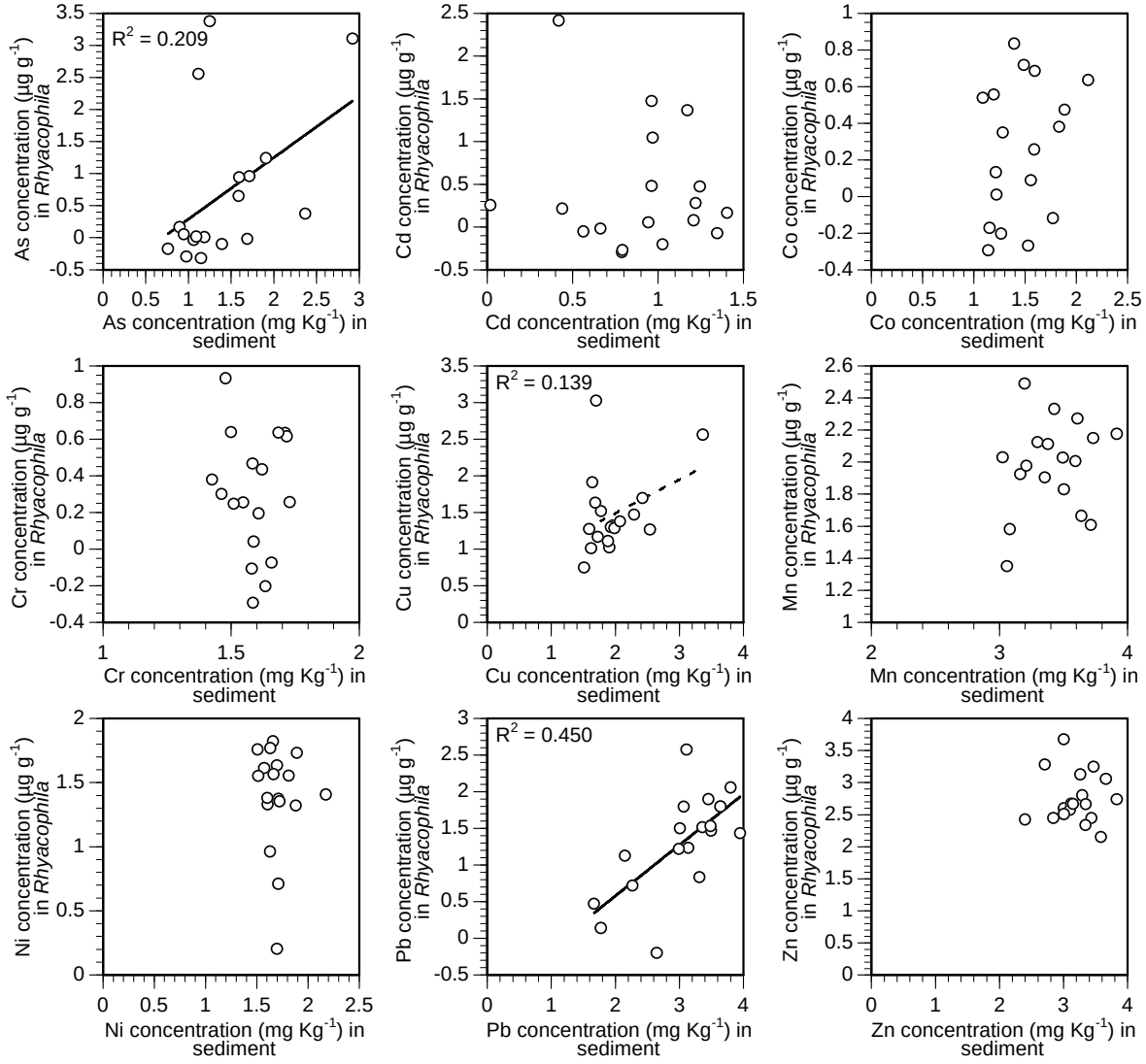


Figure 4.7d Relationships between the concentrations of metals and metalloids (As, Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn) in fine sediments (< 63 µm) collected for source apportionment and corresponding concentrations in the tissues of *Rhyacophila*. Solid lines shown where significant: dashed lines close to significant.



4.3.5 Relationships between biomonitor body burden and dissolved metal concentrations in river water

EA water chemistry monitoring data were available for 46 of the field sites. For these sites, long-term mean dissolved metal concentrations in the overlying water were calculated from the available data and compared to the body burdens of the biomonitor species, sediment metal concentrations and, for the most downstream sites in each catchment, metal concentrations in fine sediments collected for source apportionment. Here, we attempted to establish the influence of dissolved metals in river water on bioavailability measured as tissue concentrations in the biomonitor taxa, by comparing relationships with those derived for sediment metal concentrations. It should be noted that the measures of water chemistry were long-term averages based on multiple sampling occasions and, therefore, are more likely to represent an accurate characterisation of site conditions than the sediment samples collected in this project. However, water chemistry data were not available for all sites used in the field survey, reducing the statistical power to detect relationships.

Mean dissolved concentrations of copper, lead and zinc in river water were correlated with corresponding concentrations in the fine sediment and in the fine sediments (< 63 µm) collected for source apportionment. Mean dissolved concentrations of cadmium were also correlated with corresponding concentrations in the fine sediment. These results suggest that either:

a) the abandoned metal mines targeted in this project were releasing both particulate and dissolved metals into the environment, with the extent of release of both forms dependent on the extent of contamination at each site, and/or
b) metals in river water and sediment are not independent. Rather they can move between these two compartments.

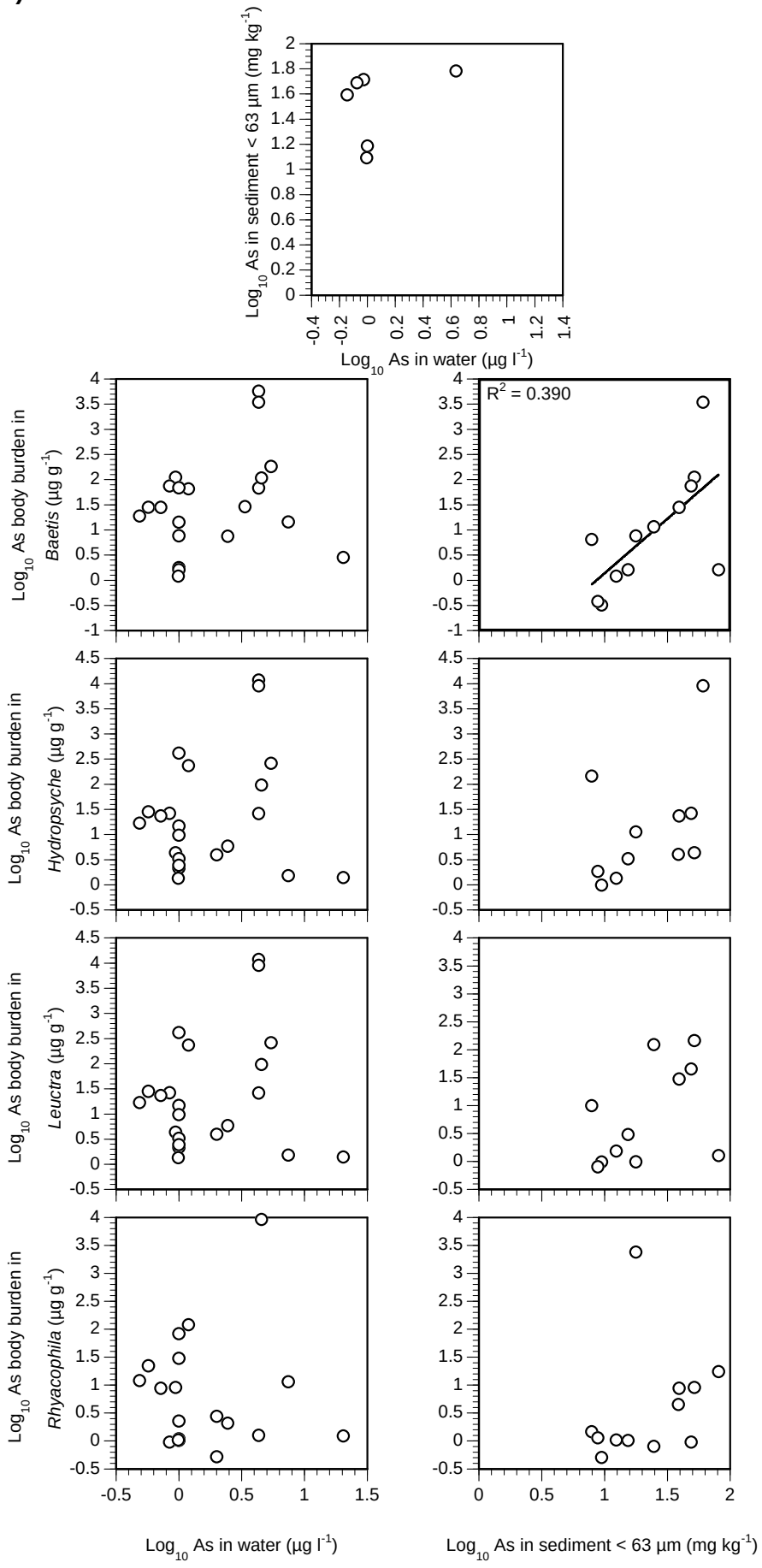
Due to the strong correlations between sediment and dissolved metal concentrations, it was not possible to determine the relative importance of sediment and water using this approach. An experimental approach (see Section 7) or a field campaign that targeted sites where abandoned mine facilities are releasing either contaminated water or sediment alone would be required to determine the relative influence of these two compartments.

Nevertheless, the body burdens of arsenic in *Baetis* was correlated with the concentration in the sediment fraction used for source apportionment (Figure 4.8a). Cadmium in *Hydropsyche* was correlated with cadmium in the sediment fraction used for source apportionment and weakly correlated with the water (Figure 4.8b). Copper in all biomonitor taxa were correlated with copper concentrations in both water and fine sediment, and in the case of *Baetis* the sediment fraction used for source apportionment (Figure 4.8c). Here the relationships with body burdens in biomonitor taxa were stronger with water than sediment for *Hydropsyche*, *Leuctra* and *Rhyacophila*, but it should be noted that relationships with the sediment copper concentrations were stronger in the full data set and particularly when the influence of environmental conditions was taken into account (see Sections 4.3.3 and 4.3.4). Lead in all the biomonitor taxa was correlated with lead in the water and both measures in the sediment, with no clear pattern between the different compartments (Figure 4.8e). Zinc in *Baetis* and *Leuctra* were correlated with concentrations in both fine sediment and water (Figure 4.8f). Nickel in *Leuctra* and *Rhyacophila* were correlated with concentrations in the sediment (Figure 4.8d).

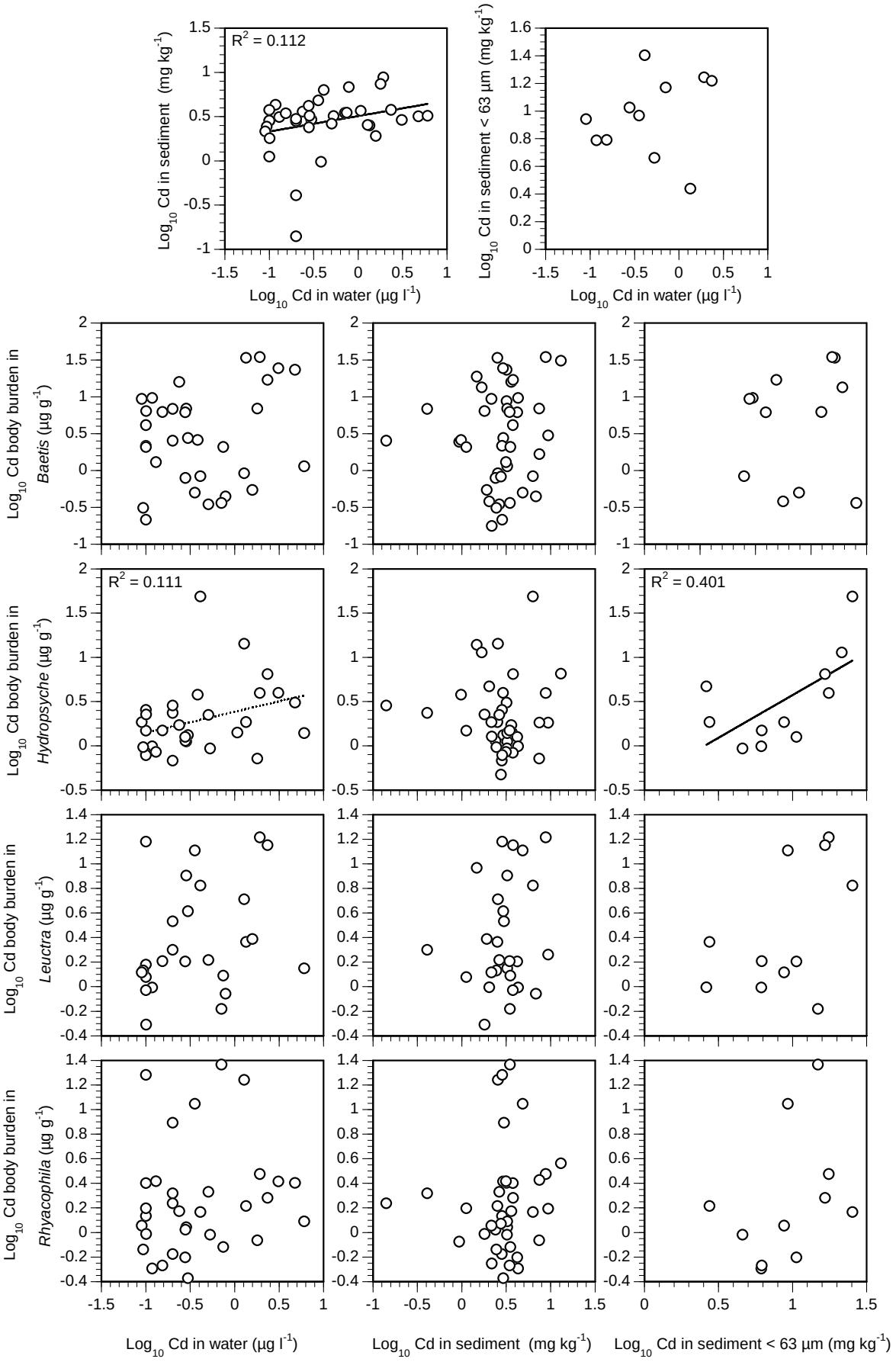
As stated above, due to the correlation between the mean dissolved metal concentrations in river water and the concentrations of metals in the sediment, this analysis was not able to draw any substantial conclusion on the relative importance of sediment and water as sources of bioavailable metal. However, at least for arsenic, cadmium and nickel, sediment appears to be a more important source than river water. The results for copper, lead, and zinc are less easy to interpret.

Figure 4.8 (overleaf) Relationships between mean concentrations of dissolved metals and metalloids (As, Cd, Cu, Ni, Pb and Zn) in river water, metal concentrations in fine sediments collected for source apportionment and corresponding concentrations in the tissues of biomonitor taxa. Solid lines shown where significant: dashed lines close to significant.

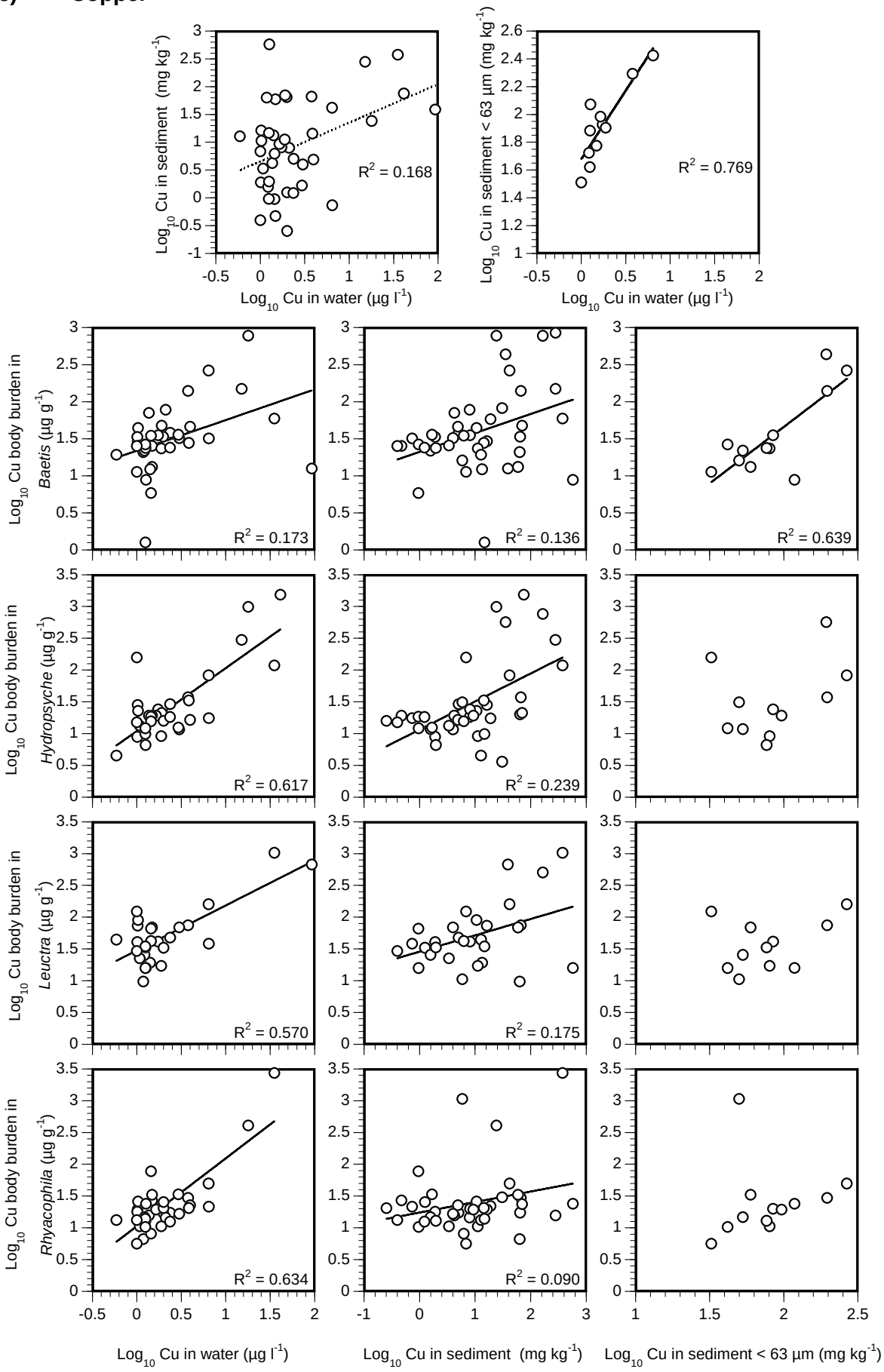
a) Arsenic


Log10 As in sediment < 63 µm (mg kg-1)
Log10 As in water (µg l-1)
R2 = 0.390
Log10 As body burden in Baetis (µg g-1)
Log10 As body burden in Hydropsyche (µg g-1)
Log10 As body burden in Leuctra (µg g-1)
Log10 As body burden in Rhyacophila (µg g-1)
Log10 As in water (µg l-1)
Log10 As in sediment < 63 µm (mg kg-1)

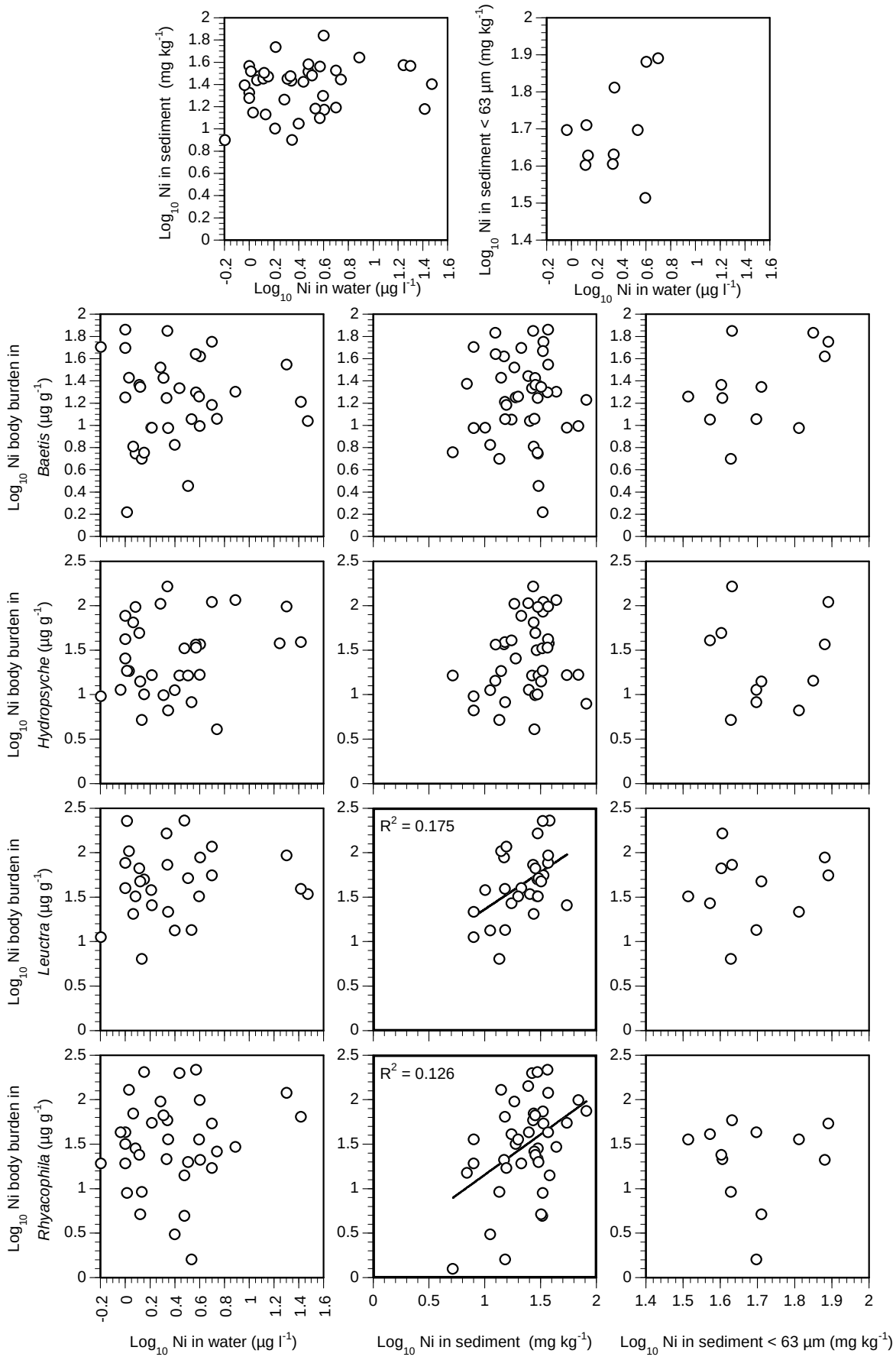
b) Cadmium


R² = 0.112
Log10 Cd in sediment (mg kg-1)
Log10 Cd in water (µg l-1)
Log10 Cd in sediment < 63 µm (mg kg-1)
Log10 Cd body burden in Baetis (µg g-1)
Log10 Cd body burden in Hydropsyche (µg g-1)
R² = 0.111
R² = 0.401
Log10 Cd body burden in Leuctra (µg g-1)
Log10 Cd body burden in Rhyacophila (µg g-1)
Log10 Cd in water (µg l-1)
Log10 Cd in sediment (mg kg-1)
Log10 Cd in sediment < 63 µm (mg kg-1)

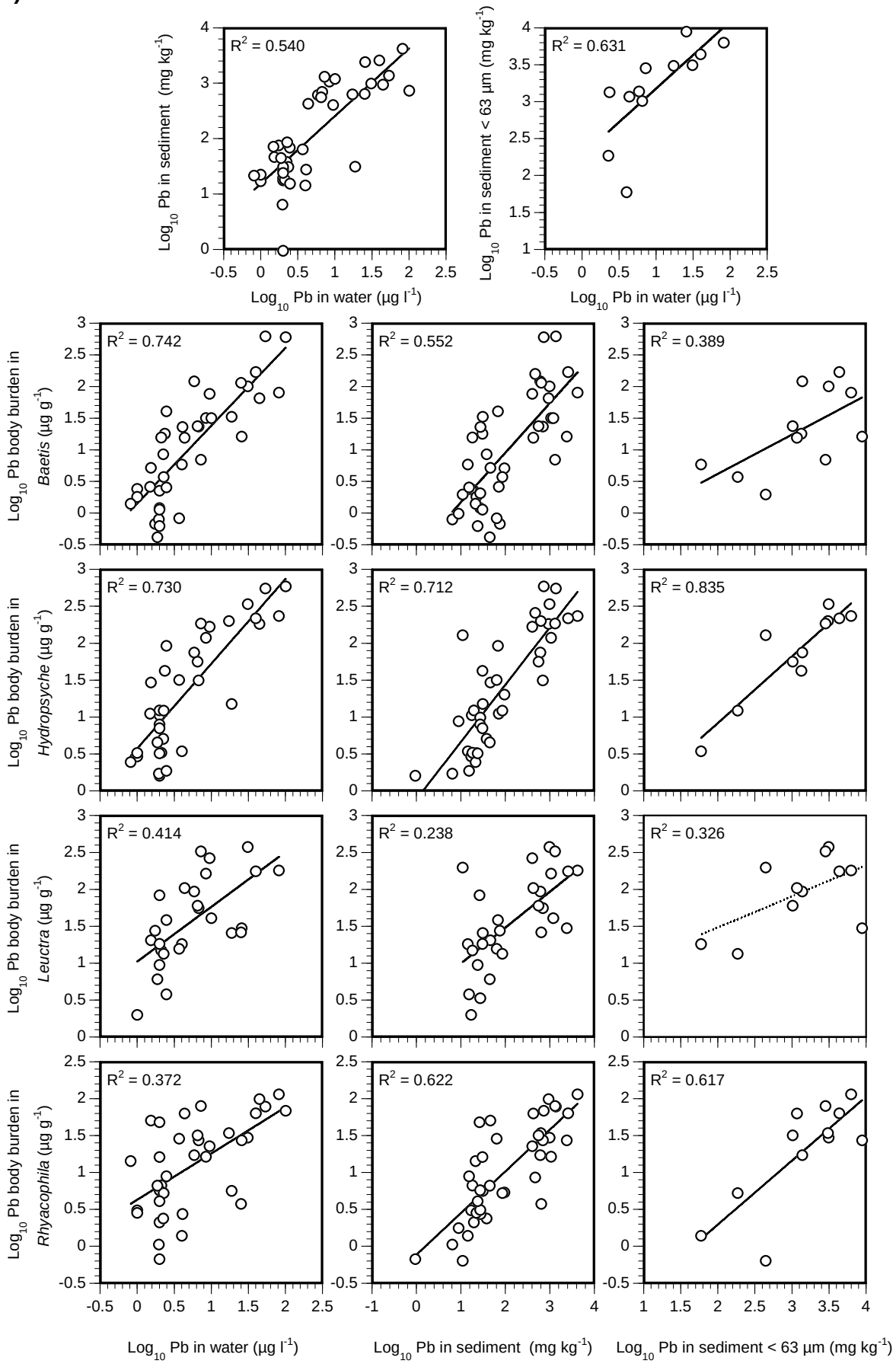
c) Copper


Log10 Cu in sediment (mg kg-1)
R2 = 0.168
Log10 Cu in water (µg l-1)
Log10 Cu in sediment < 63 µm (mg kg-1)
R2 = 0.769
Log10 Cu in water (µg l-1)
Log10 Cu body burden in Baetis (µg g-1)
R2 = 0.173
R2 = 0.136
R2 = 0.639
Log10 Cu body burden in Hydropsyche (µg g-1)
R2 = 0.617
R2 = 0.239
Log10 Cu body burden in Leuctra (µg g-1)
R2 = 0.570
R2 = 0.175
Log10 Cu body burden in Rhyacophila (µg g-1)
R2 = 0.634
R2 = 0.090
Log10 Cu in water (µg l-1)
Log10 Cu in sediment (mg kg-1)
Log10 Cu in sediment < 63 µm (mg kg-1)

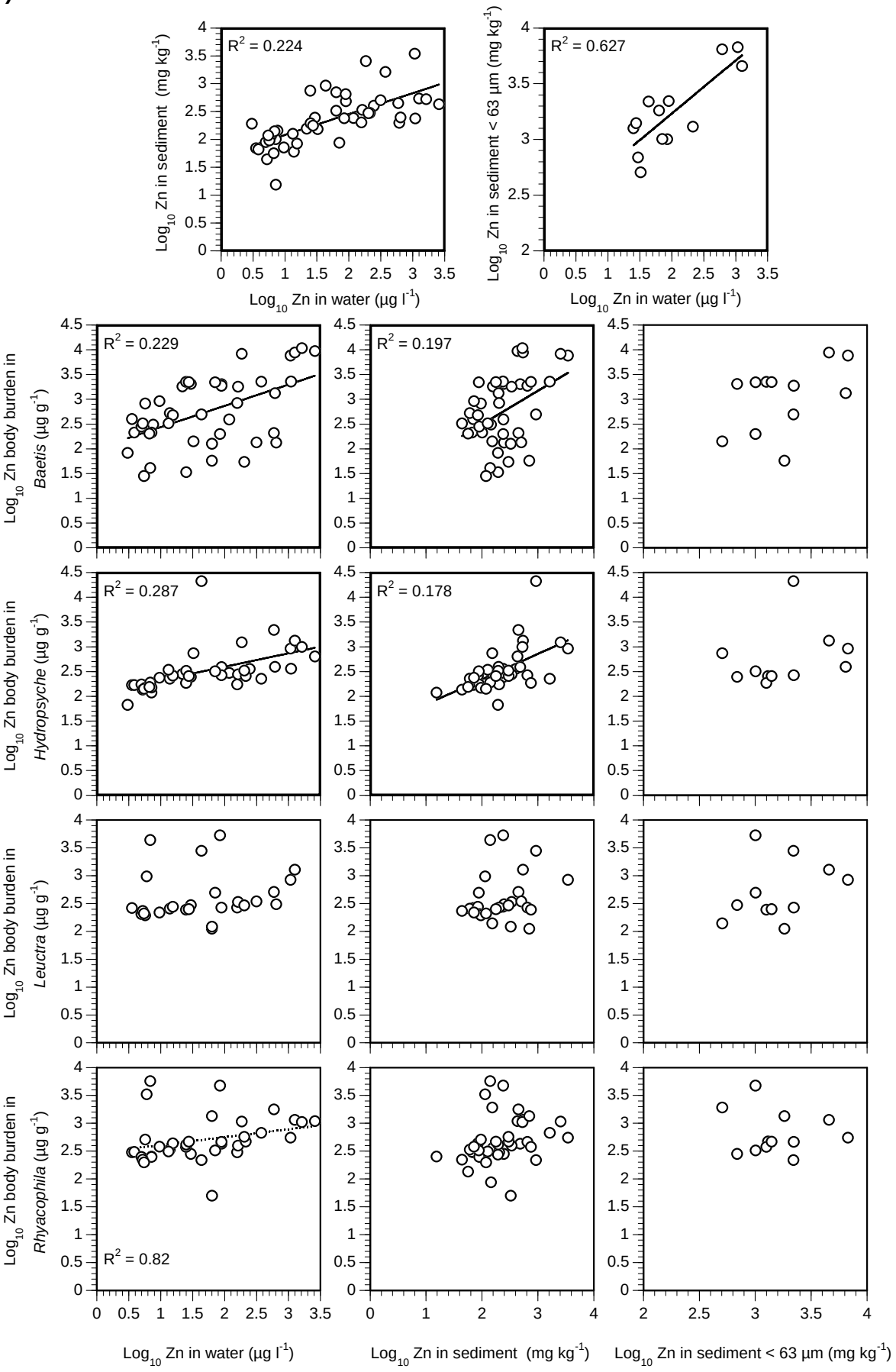
d) Nickel


Log10 Ni in sediment (mg kg-1)
Log10 Ni in water (µg l-1)
Log10 Ni in sediment < 63 µm (mg kg-1)
Log10 Ni body burden in Baetis (µg g-1)
Log10 Ni body burden in Hydropsyche (µg g-1)
Log10 Ni body burden in Leuctra (µg g-1)
Log10 Ni body burden in Rhyacophila (µg g-1)
R2 = 0.175
R2 = 0.126

e) Lead


R² = 0.540
Log10 Pb in sediment (mg kg-1)
Log10 Pb in water (µg l-1)
R² = 0.631
Log10 Pb in sediment < 63 µm (mg kg-1)
Log10 Pb in water (µg l-1)
R² = 0.742
R² = 0.552
R² = 0.389
Log10 Pb body burden in Baetis (µg g-1)
R² = 0.730
R² = 0.712
R² = 0.835
Log10 Pb body burden in Hydropsyche (µg g-1)
R² = 0.414
R² = 0.238
R² = 0.326
Log10 Pb body burden in Leuctra (µg g-1)
R² = 0.372
R² = 0.622
R² = 0.617
Log10 Pb body burden in Rhyacophila (µg g-1)
Log10 Pb in water (µg l-1)
Log10 Pb in sediment (mg kg-1)
Log10 Pb in sediment < 63 µm (mg kg-1)

f) Zinc


R² = 0.224
Log10 Zn in sediment (mg kg-1)
Log10 Zn in water (µg l-1)
R² = 0.627
Log10 Zn in sediment < 63 µm (mg kg-1)
Log10 Zn in water (µg l-1)
R² = 0.229
Log10 Zn body burden in Baetis (µg g-1)
R² = 0.197
R² = 0.287
Log10 Zn body burden in Hydropsyche (µg g-1)
R² = 0.178
Log10 Zn body burden in Leuctra (µg g-1)
Log10 Zn body burden in Rhyacophila (µg g-1)
R² = 0.82
Log10 Zn in water (µg l-1)
Log10 Zn in sediment (mg kg-1)
Log10 Zn in sediment < 63 µm (mg kg-1)

4.3.6 Relationships between biomonitor body burden and species richness

Following the model of uptake of metals described in Section 1, it was decided to use quantile regression with a threshold response model (as described in Section 3.1) to determine if bioavailability of metals as measured using the body burden of the biomonitor taxa (as described in Section 4.3.1) was related to changes in taxon richness in the invertebrate samples collected from the same site. Here, again, we expected taxon richness to show a significant decline once a threshold bioavailability of metal was exceeded. As before, significance was determined using AIC values calculated using the method described in Pacheco et al. (2005). The threshold model was selected as optimal if it had the lowest AIC values compared with an exponential, a linear and a null model.

Baetis provided optimal fits for arsenic, cadmium, copper and zinc, and a close to optimal fit for lead. However, the threshold for the model with zinc was near to the upper limit of the range of body burden measured and may be trivial (i.e. the relationship may be highly influenced by the few points above the threshold). *Hydropsyche* provided optimal fits for copper and nickel, and a close to optimal fit for lead. *Leuctra* provided optimal fits for arsenic, cadmium, copper, nickel and zinc. *Rhyacophila* only provided a close to optimal fit for lead. In each case where lead provided a close to optimal model, the linear model was the other selected model, which is consistent with the relatively low threshold bioavailability for lead detected for all three species.

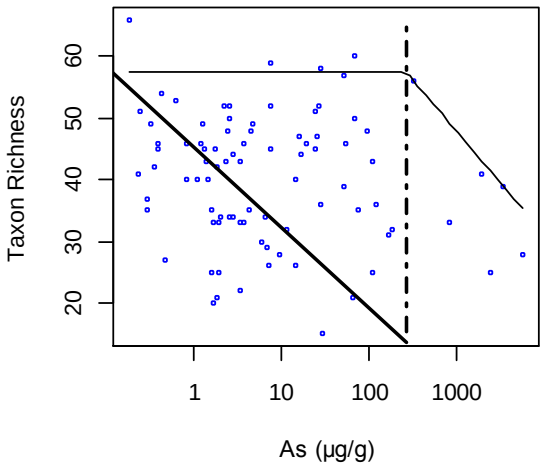
These results confirm that the biomonitor approach can be used to assess the bioavailability of metals from sediment at ecologically significant concentrations. This may allow regulatory agencies to survey possible risks at sites simply by analyzing body burdens in certain species and comparing measured metal residues with those corresponding to undamaged conditions (i.e. no community level impact).

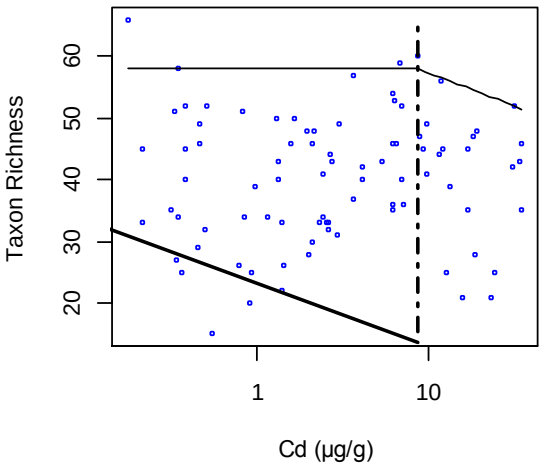
Table 4.10 Summary of results from quantile regression showing modelled thresholds in taxon richness of invertebrates in relation to the body burden (µg g^{-1}) of arsenic, cadmium, copper nickel, lead and zinc for the biomonitor species B*aetis, Hydropsyche, Leuctra* and *Rhyacophila* collected from the same site. Figures shown are from model fits that were optimal based on AIC criteria: figures in red are from models that were close to optimal, figures in italics are potentially trivial.

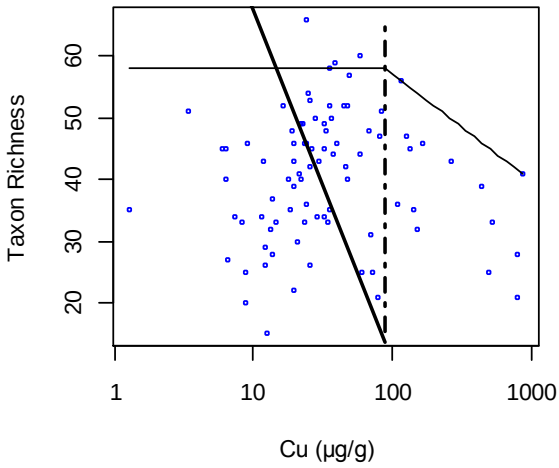
	Baetis	*Hydropsyche*	*Leuctra*	*Rhyacophila*
As	274		210	
Cd	8.8		7.7	
Cu	89	29	80	
Ni		86	80	
Pb	8.5	24		1.8
Zn	*6480*		294	

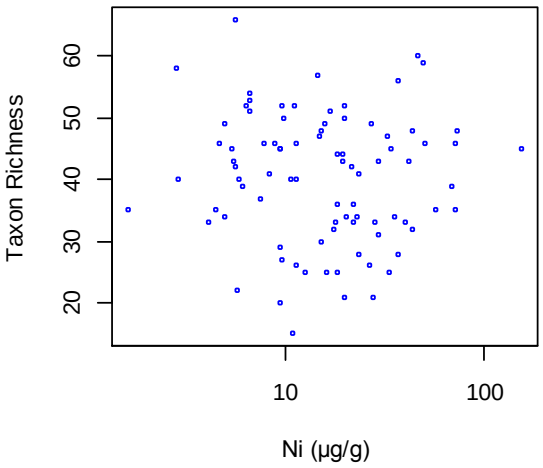
Figure 4.9 (overleaf) Relationships between taxon richness of invertebrates and the body burden (µg g^{-1}) of arsenic, cadmium, copper nickel, lead and zinc for the biomonitor species A) *Baetis,* B) *Hydropsyche,* C) *Leuctra* and D) *Rhyacophila* collected from the same site, as modelled using quantile regression with a threshold response. Lines are shown for optimal or close to optimal models.

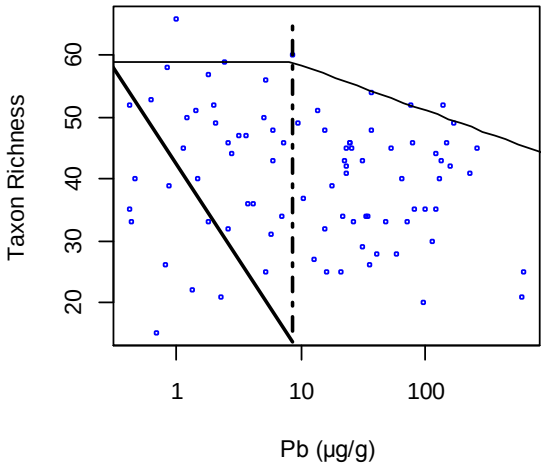
Baetis

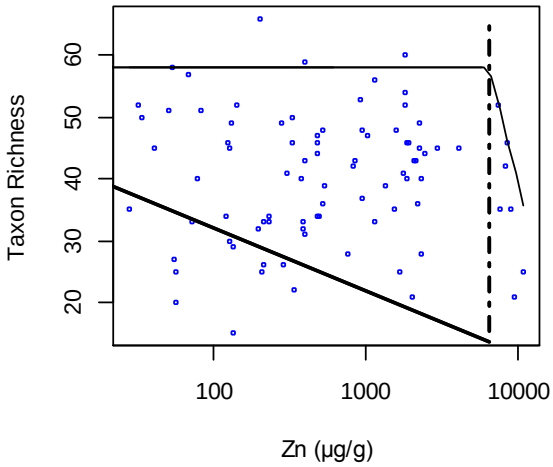

Taxon Richness
20
30
40
50
60
1
10
100
1000
As (µg/g)


Taxon Richness
20
30
40
50
60
1
10
Cd (µg/g)

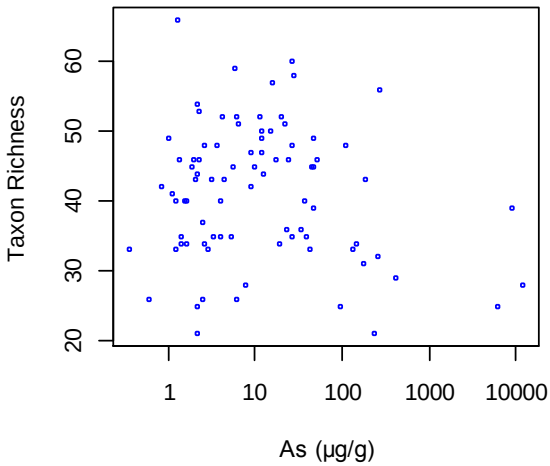

Taxon Richness
20
30
40
50
60
1
10
100
1000
Cu (µg/g)

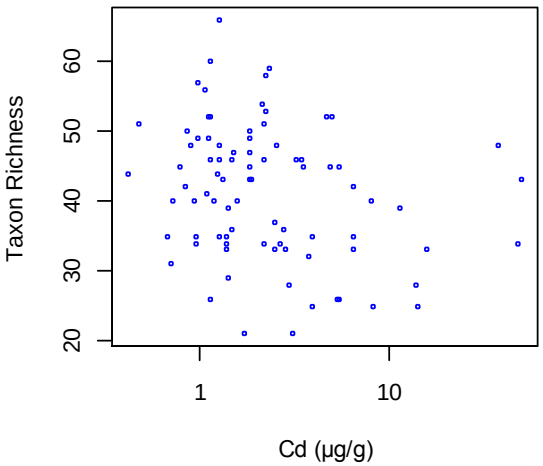

Taxon Richness
20
30
40
50
60
10
100
Ni (µg/g)

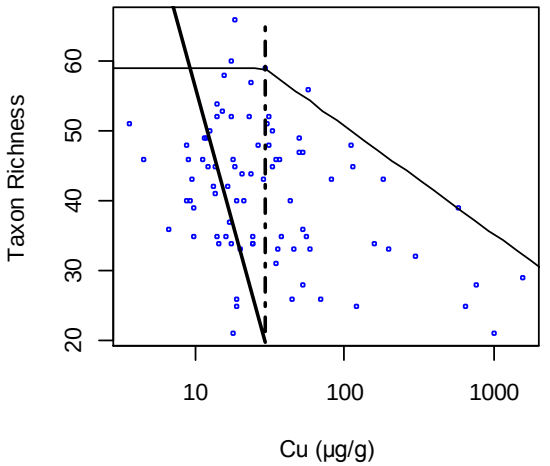

Taxon Richness
20
30
40
50
60
1
10
100
Pb (µg/g)

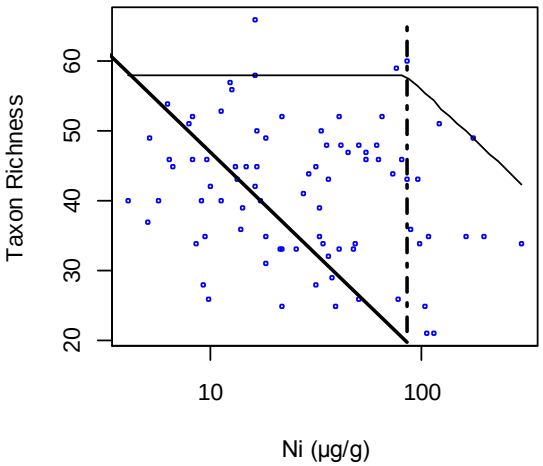

Taxon Richness
20
30
40
50
60
100
1000
10000
Zn (µg/g)

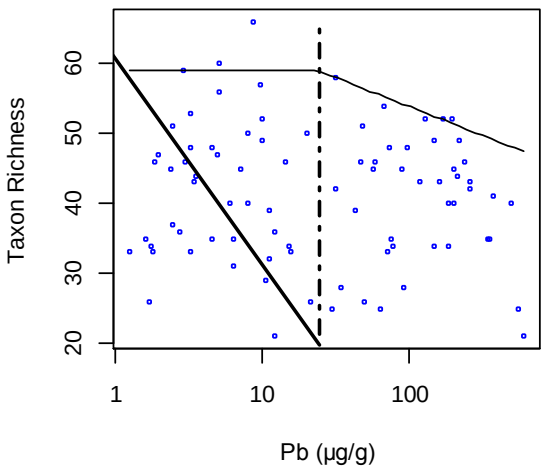
Hydropsyche

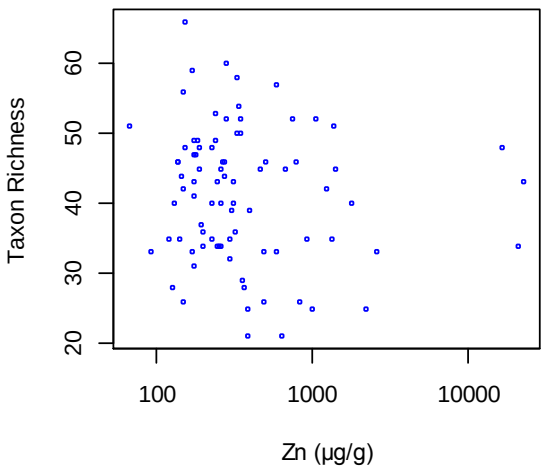




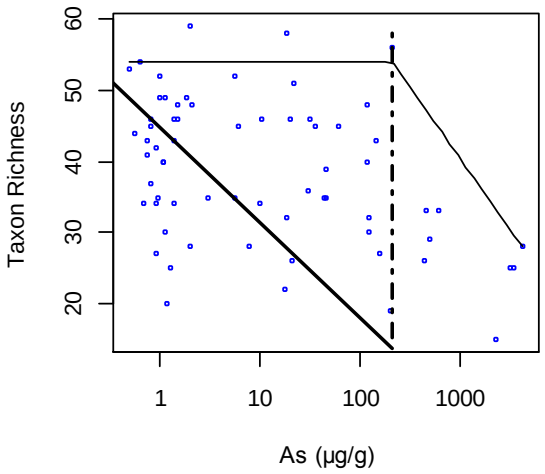


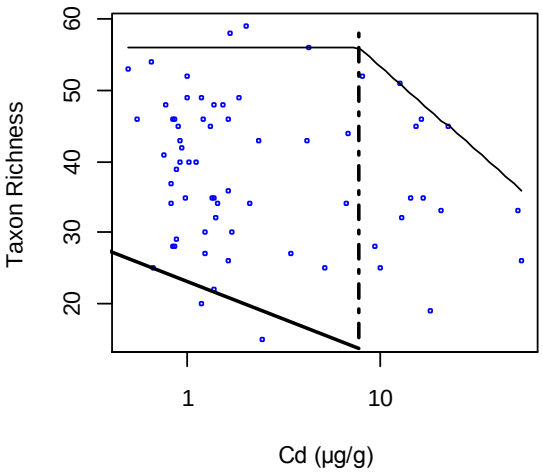


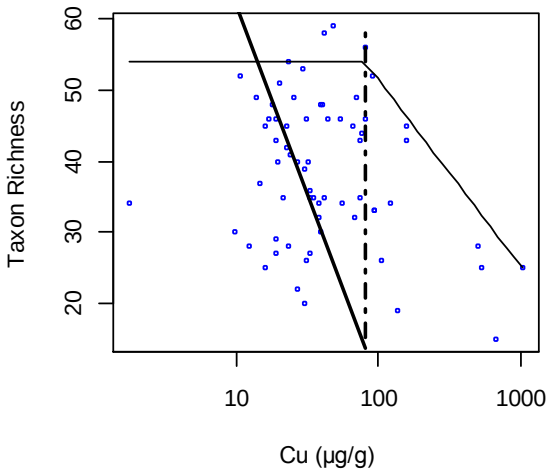


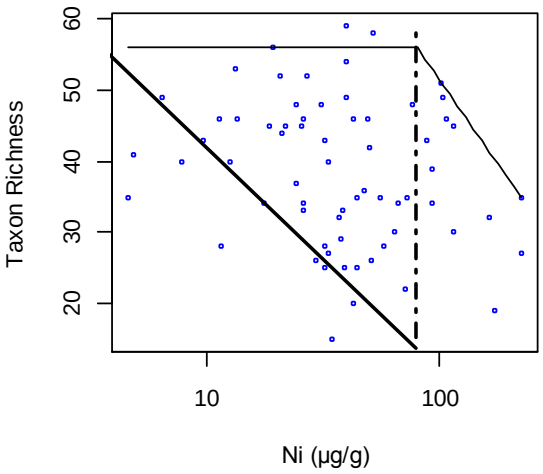


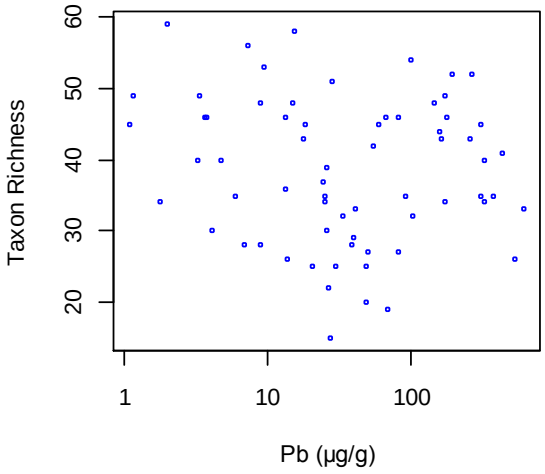
Leuctra

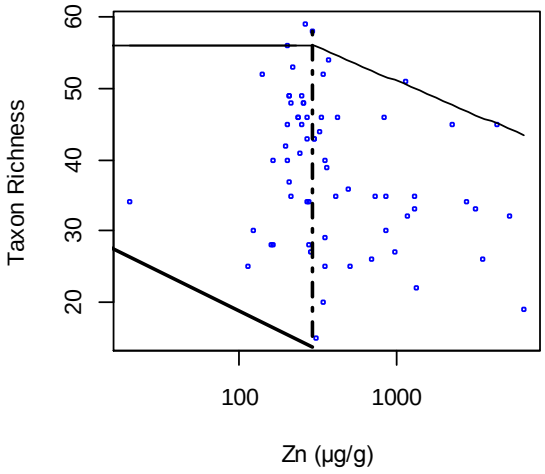

Taxon Richness
20
30
40
50
60
1
10
100
1000
As (µg/g)


Taxon Richness
20
30
40
50
60
1
10
Cd (µg/g)

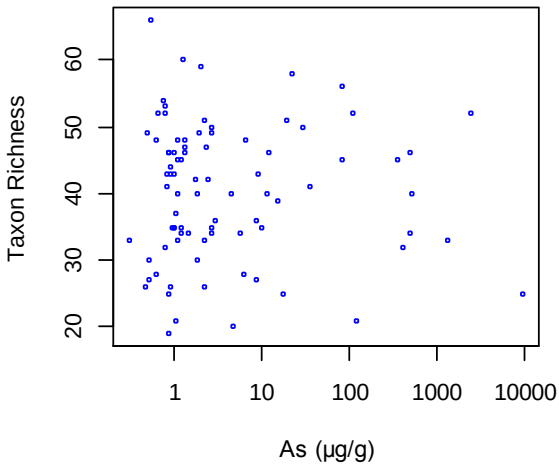

Taxon Richness
20
30
40
50
60
10
100
1000
Cu (µg/g)

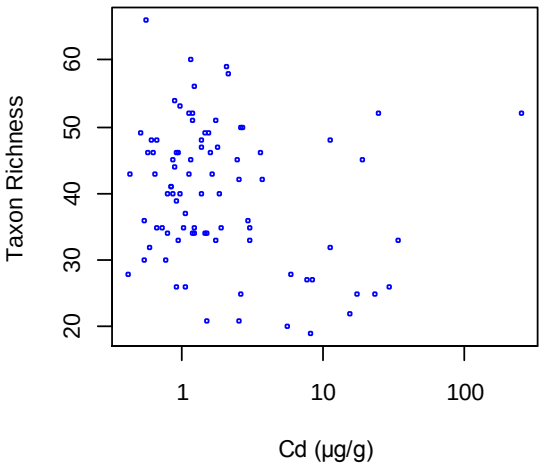

Taxon Richness
20
30
40
50
60
10
100
Ni (µg/g)

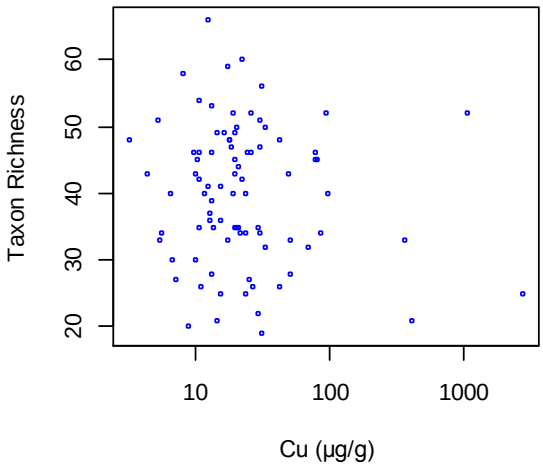

Taxon Richness
20
30
40
50
60
1
10
100
Pb (µg/g)

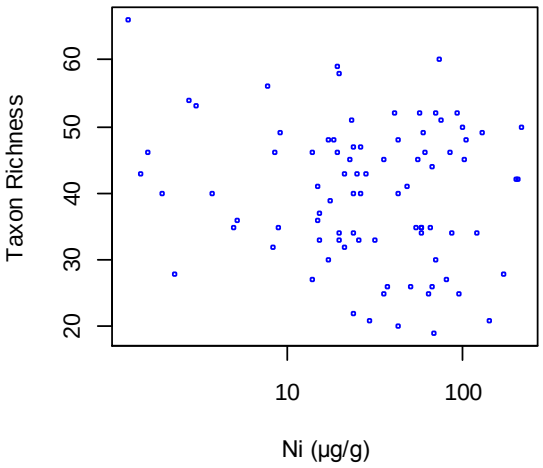

Taxon Richness
20
30
40
50
60
100
1000
Zn (µg/g)

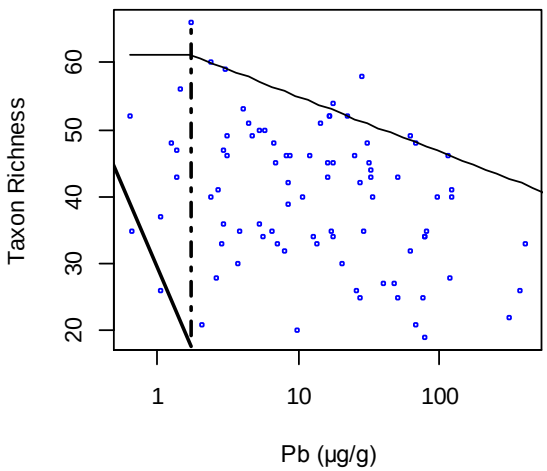
Rhyacophila

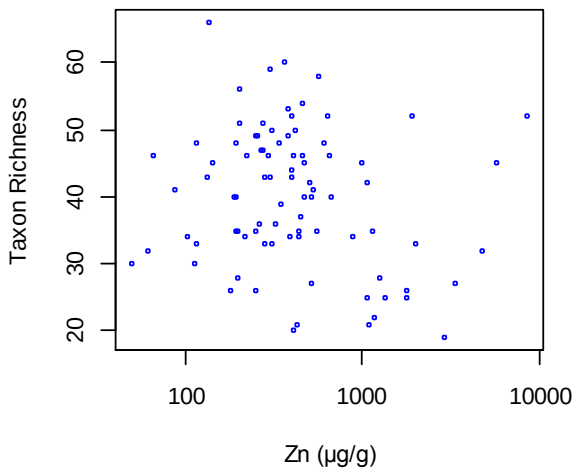












The relationship between between biomonitor body burden and sediment metal concentrations (Section 4.3.4c) was used to determine the sediment metal concentrations at which the biomonitor body burdens indicated a threshold in taxon richness of invertebrates (Table 4.10 and Figure 4.9). Here the threshold in biomonitor body burden identified using quantile regression was used to calculate sediment metal concentrations using the equations in Table 4.11 (from Figures 4.6 and 4.7). Using this two-step procedure, the influence of bioavailability of sediment metals is more accurately represented than if sediment concentrations were directly related to taxon richness of invertebrates. However, this approach introduces more uncertainty, as the result is dependent upon two relationships each with their associated uncertainty.

Table 4.11 Summary of sediment metal concentrations derived from quantile regression modelled thresholds in taxon richness of invertebrates in relation to the body burden (µg g^{-1}) for the biomonitor species collected from the same site.

	Baetis	*Hydropsyche*	*Leuctra*	*Rhyacophila*	Mean
As	372 [a]		396 [a]		384 [a]
Cd	–		54 [b]		54 [b]
Cu	135	21	70		75
Ni		537 [b]	231		384
Pb	65	96		1.5	54 [c]
Zn	*517,176*		1,561		1,561 [d]

Figures calculated from relationships between sediment metal concentrations and biomonitor body burden where model fits were optimal based on AIC criteria: figures in red are from models that were close to optimal, figures in italics are potentially trivial.
– No significant relationship between sediment and biomonitor body burden
[a] based on relationship with fine sediment <63 µm used for source apportionment.
[b] based on weak relationship between sediment and biomonitor body burden
[c] based on threshold model that was close to optimal.
[d] threshold model that was trivial not included.

4.3.7 Community response

The aim of the biomonitor analysis is to provide a measure of local bioavailability of metals at sites contaminated by mining, that can then be related to the ecological responses of the benthic macroinvertebrate community, either directly within or in contact with the sediment or via sediment exchange with the water column. With these data we will address **Objective 4a** [**Establish the sensitivity of benthic invertebrate species to metal bioavailability (using partial ordination techniques) and, on that basis, devise a new diagnostic index that can be used to determine failure of test sites as a consequence of contaminated sediments**.]

For each of the 99 stream sites sampled we had data describing the benthic macroinvertebrate community, the physicochemical characteristics of the sampled reach (Table 4.12) , and the metal body burden of *Baetis* spp., *Hydropsyche* spp., *Leuctra* spp. and *Rhyacophila* spp. (see Section 4.2 for description of sampling and laboratory methods).

Table 4.12 Environmental variables used to account for natural background variation in the sampled stream macroinvertebrate community, showing the independent explanatory power of each variable (marginal effect), and the additional contribution of each successive variable to the forward selected model (conditional effect).

Environmental variable	Units	Minimum (untransformed)	Maximum (untransformed)	Marginal fit	Conditional fit
Distance from source	log km	1.05	24.1	0.06	0.06
Elevation	m	7	425	0.17	0.17
Stream slope	log m km^{-1}	0.9	66	0.10	0.04
Stream width	log m	1.5	19.6	0.07	-
Stream depth	log cm	4.3	67.3	0.05	0.05
Substrate phi score[1]	-	-7.3	0.95	0.11	0.03
pH	-	4.2	8.77	0.05	0.03
Conductivity	log μS cm^{-1}	23	736	0.09	0.07
Organic fine sediment content	Arcsine mean %	3.4	34.8	0.03	-
Mass of calcium in fine sediment	log mg m^{-2}	1.02	4.67	0.06	0.02

[1] Substrate phi score is the weighted sum of percent cover values for different substrate size categories: phi score = -7.75(% cover of boulders and cobbles) -3.25(% cover of pebbles and gravel)+2(% cover sand) + 8(% cover of silt and clay).

Across the 99 sites we recorded the abundance of 196 discrete taxa (see Appendix 5). Taxon richness varied from 15 (River Hayle, downstream of Godolphin Bridge Mine) to 66 (Linburn Beck, independent control stream in Weardale). Abundance of individuals captured in a sample varied widely from 44 (R. Wye, downstream of Nantiago Mine) to 7067 (Porthleven Stream, upstream of Great Fortune Mine).

The specific objective of the field survey was to quantify the association between variation in the macroinvertebrate assemblage and the metals stressor gradient having first factored out that portion of the biological variation correlated with natural background variation among streams. From such an analysis, the relative sensitivity of a range of macroinvertebrates to

metals stress could be quantified and would form the empirical basis for a new diagnostic biotic index.

Multivariate ordination was used to first quantify variation in the macroinvertebrate assemblage, and then to determine which set of natural environmental variables best described the pattern. Of the 196 recorded taxa, 106 occurred in fewer than 10 samples and therefore were excluded to ensure that inferences about sensitivities to metal pollution were based on a reasonable number of replicate occurrences. An initial detrended correspondence analysis on the matrix of log-transformed abundance data for 90 taxa across 99 samples found that that taxa turnover (DCA axis 1 gradient length = 2.41) was sufficient to meet the unimodal response assumption of canonical correspondence analysis (CCA) (ter Braak, 1994). A CCA of the biotic data against the environmental variables (Table 4.12) was undertaken with Hill's scaling of ordination scores with focus on inter-species distances, and manual forward selection (n = 999 permutations, $p \leq 0.01$ as the significance threshold for inclusion in the model) to determine the optimal subset of predictor variables that accounted for natural background gradients in the macroinvertebrate assemblage. The 10 variables were ranked according to their independent ability to account for variation in the biological data. The highest ranking variable was added to the model and then remaining variables were again ranked according to their independent ability to account for the subsequent residual variation in the biological data. This process continued until the addition of another variable would not lead to a significant gain in the explanatory power of the model. The most parsimonious explanatory model included elevation, conductivity, distance from source, depth, slope, pH, substrate composition and mass of calcium in stream bed fine sediment (Table 4.12). Width and fine sediment organic content were not selected.

A partial CCA (pCCA) was then carried out with the selected environmental variables describing the natural background characteristics of the sites considered as co-variables in the analysis. Residual variation in the macroinvertebrate assemblage, having factored out that associated with the co-variables, was then related to measures of metal bioavailability at each site i.e. arsenic, cadmium, copper, nickel, lead and zinc body burden. Not all biomonitor groups were present at all catchments or sites (Table 4.13). *Baetis* and *Rhyacophila* were the most widespread biomonitors with specimens collected from 86 of the 99 sites. *Gammarus* were collected from only 30 sites. *Baetis* tended to be more abundant at sites than the equally widespread *Rhyacophila* and hence was chosen as the group to represent the level of metal bioavailability at each site in the pCCA. For the 13 sites where *Baetis* was absent, a modelled tissue concentration for each metal (arsenic, cadmium, copper, nickel, lead and zinc) was derived based on statistically significant relationships between *Baetis* metal tissue concentration and that of *Hydropsyche* for missing sites in Catchments A, I and J, *Leuctra* for missing sites in Catchments K, Q and T and *Rhyacophila* for missing Sites in Catchments Q and T (Appendix 5). Where possible, regressions were calculated for missing sites based only on data from other sites in the same region, e.g. for Catchment I *Baetis*-*Hydropsyche* regressions were based on data from other Welsh catchments.

The pCCA of the biotic data against the *Baetis* metal body burden variables was undertaken with Hill's scaling of ordination scores with focus on inter-species distances, and manual forward selection (n = 999 permutations, $p \leq 0.01$ as the significance threshold for inclusion in the model) to determine the optimal subset of metals that could account for variation in the macroinvertebrate assemblage across the metal stress gradients

Table 4.13. Number of sites within each catchment where specimens of each of the five genera were collected for metal body burden analysis.

Catchment	*Baetis*	*Hydropsyche*	*Leuctra*	*Rhyacophila*	*Gammarus*
A	3	4	2	3	2
B	5	5	3	3	1
C	5	5	3	1	1
D	5	3	2	3	3
E	5	5	1	4	5
F	5	5	1	5	2
G	5	5	5	5	-
H	5	5	3	5	-
I	2	5	5	5	-
J	2	5	-	5	-
K	1	-	5	4	-
L	5	5	1	5	5
M	5	1	5	5	2
N	5	5	5	5	1
O	5	3	5	3	-
P	5	5	1	5	5
Q	4	4	5	5	-
R	5	5	5	5	-
S	5	5	4	5	2
T	4	4	5	5	1
Total	86	84	66	86	30

Table 4.14. *Baetis* metal tissue concentration variables used to account for residual variation in the sampled stream macroinvertebrate community, showing the independent explanatory power of each variable (marginal effect), and the additional contribution of each successive variable to the forward selected model (conditional effect).

Metal concentration in Baetis	Units	Minimum μg g^{-1} (untransformed)	Maximum μg g^{-1} (untransformed)	Marginal fit	Conditional fit
Arsenic	log μg g^{-1}	0.18	5734	0.02	-
Cadmium	log μg g^{-1}	0.18	35.05	0.02	-
Copper	log μg g^{-1}	1.27	1008	0.02	0.02
Nickel	log μg g^{-1}	1.66	153.5	0.03	0.03
Lead	log μg g^{-1}	0.41	620.6	0.04	0.04
Zinc	log μg g^{-1}	28.2	10859	0.02	-

The pCCA model selected included lead, nickel, and copper, with arsenic, cadmium or zinc not making a significant additional contribution to the model (Table 4.13). The three constrained axes in the ordination accounted for 45%, 32% and 22% respectively of the pCCA biology-metals relationship. Axis 1 was most strongly correlated with the lead body burden (r = 0.67), axis 2 with nickel body burden (r = 0.71) and axes 3 with Cu (r = 0.63)

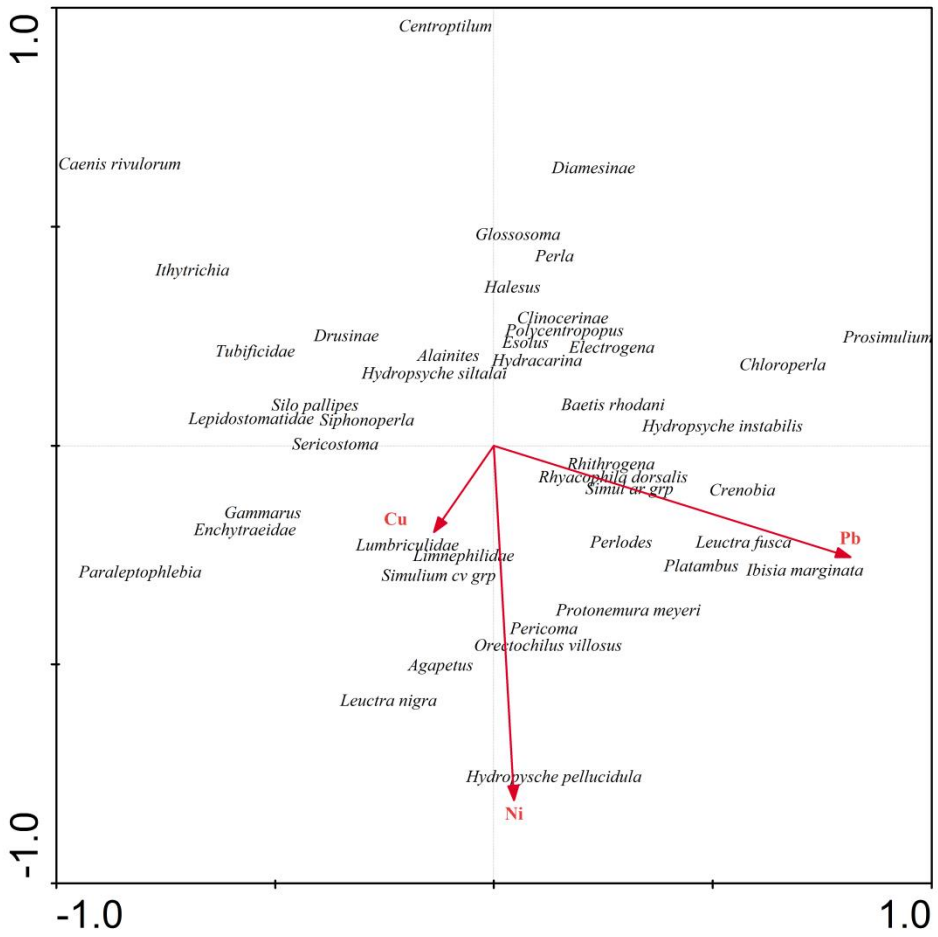
(Table 2.14, Figure 4.10). The position of taxa (their pCCA species scores) along the three constrained axes of the pCCA ordination space provide a robust ranking of taxa in terms of their association with the three metal body burden gradients, without the confounding influence of other measured environmental factors. The pCCA species scores are calculated as the weighted average of the sample scores and indicate the centre of the taxon distribution (its mode, assuming a Gaussian response curve) along each constrained ordination axis (ter Braak and Šmilauer, 2002). For each axis in turn, tolerance scores from 0 to 100 were allocated to taxa based on their relative position along the axis, with the most metal-tolerant species being scored 0 and taxa successively more distant along the axis being assigned scores equivalent to the percent of the axis distance between the highest and lowest scoring taxa (Table 4.15). The tolerance scores for each taxon for the three axes were combined as a weighted sum, with the weights being the proportion of the variance in the pCCA biology-metals relationship accounted for by each axis (0.45, 0.32 and 0.22 respectively). The weighted-sum of tolerance scores were converted to the percent of the distance between the highest and lowest scoring taxa, as before, such that the water snipe fly *Ibisia marginata* scored zero as the taxon most associated with high metal concentrations in *Baetis,* and the mayfly *Caenis rivulorum* scored 100 as the taxon most associated with low metal concentrations in *Baetis* (Table 4.16).

Table 4.15. Correlation coefficients between metal bioavailability variables and the three constrained pCCA axes.

Metal bioavailability	pCCA axis 1	pCCA axis 2	pCCA axis 3
Cu in *Baetis*	-0.146	-0.200	**0.625**
Ni in *Baetis*	0.043	**-0.712**	-0.022
Pb in *Baetis*	**0.665**	-0.197	0.242

Other taxa associated with high levels of metals include the beetles *Orectochilus villosus* and *Platambus maculatus*, the stoneflies *Protonemura meyeri*, *Chloroperla tripunctata*, *Leuctra fusca* and *L. hippopus*, the caddis fly *Hydropsyche pellucidula*, the dragonfly *Cordulegaster boltoni*, the blackfly *Prosimulium* and the flatworms *Crenobia alpina* and *Phagocata vitta*. At the other end of the gradient are taxa associated with low levels of metals including the caddis flies *Ithytrichia*, Drusinae, *Glossosoma*, Lepidostomatidae and *Silo pallipes*, the mayflies *Paraleptophlebia* and *Centroptilum luteolum*, the freshwater shrimp *Gammarus pulex*, the worms Tubificidae and Enchytraeidae, and the gastropod snail *Potamopyrgus antipodarum*. There was a distinct pattern of decreasing taxon richness along the first pCCA axis, correlated with increasing lead concentrations in *Baetis*. Such a gradient was not as strong along the second or third axes.

a)



a)

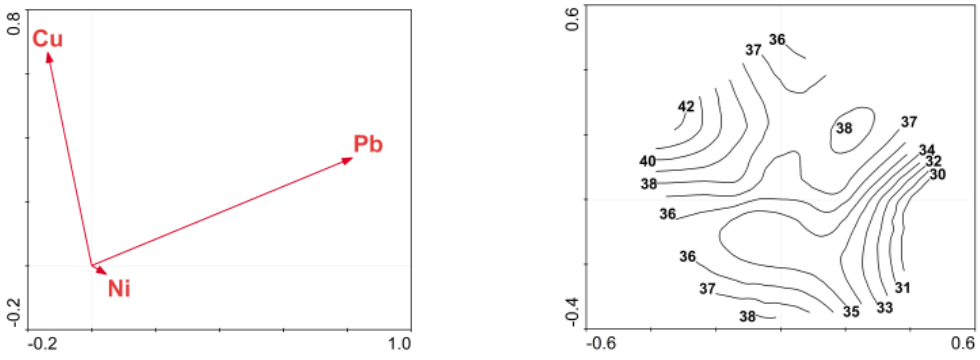


Figure 4.10 a) Partial canonical correspondence analysis biplot illustrating the positions of the 44 (out of 90) macroinvertebrate taxa with the greatest fit to the ordination axes. b) The direction of influence of the three selected explanatory variables along axes 1 and 2, and axes 1 and 3. c) The taxon richness gradient in the ordination space.

Table 4.16. The assignment of taxon scores for the species-level Metal Tolerance index (MetTol). Also presented are the pCCA axes species scores that form the basis for the ranking of taxa, and the percentile data that was used to derive the MetTol scores for each taxon.

TAXON NAME	pCCA axis 1 score	%	pCCA axis 2 score	%	pCCA axis 3 score	%	Weighted Sum of %	MetTol score
Ibisia marginata	0.5587	20	-0.2434	30	0.2994	34	26.37	0.0
Cordulegaster boltonii	0.0842	50	-0.1007	39	0.724	0	35.25	14.6
Platambus maculatus	0.4582	26	-0.2451	30	-0.0562	63	35.65	15.3
Hydropsyche pellucidula	0.0694	51	-0.7486	0	0.0195	57	35.76	15.4
Protonemura meyeri	0.307	36	-0.3354	25	0.0051	58	37.17	17.8
Orectochilus villosus	0.1243	47	-0.4155	20	0.208	42	37.25	17.9
Chloroperla tripunctata	0.543	21	0.1448	54	0.1265	48	37.49	18.3
Prosimulium sp.	0.8665	0	0.2259	58	-0.3098	83	37.54	18.4
Leuctra fusca	0.4436	27	-0.1795	34	-0.1014	67	38.16	19.4
Crenobia alpina	0.4742	25	-0.061	41	-0.1167	68	39.85	22.2
Phagocata vitta	0.188	43	-0.2045	33	0.0925	51	41.59	25.0
Serratella ignita	0.1955	43	-0.1225	38	0.0871	51	43.06	27.4
Leuctra hippopus	0.186	44	-0.1985	33	0.0141	57	43.17	27.6
Diplectrona felix	0.168	45	-0.032	43	0.182	44	43.91	28.8
Perlodes microcephalus	0.2019	42	-0.1793	34	-0.0415	62	44.09	29.1
Hydropsyche instabilis	0.342	34	0.0376	47	-0.1237	68	45.73	31.8
Polycentropus sp.	0.1495	46	0.226	58	0.3843	27	45.82	32.0
Plectrocnemia sp.	-0.0496	59	-0.2271	31	0.1741	44	46.56	33.2
Hemerodrominae	0.1105	48	0.0409	47	0.2036	42	46.60	33.3
Oulimnius tuberculatus	0.0015	55	-0.1962	33	0.1188	49	46.68	33.4
Simulium (Simulium) argyreatum group	0.1875	43	-0.0483	42	-0.0546	63	47.29	34.4
Simulium (Nevermannia) cryophilum-vernum group	-0.069	60	-0.2434	30	0.1416	47	47.39	34.6
Agapetus sp.	-0.1212	63	-0.4611	17	-0.0136	60	47.47	34.7
Pisidium sp.	0.1066	49	-0.021	44	0.0766	52	47.80	35.2
Leuctra nigra	-0.2404	71	-0.5416	12	0.0572	54	48.08	35.7
Ceratopogonidae	0.2296	41	0.0494	48	-0.0787	65	48.40	36.2
Rhyacophila dorsalis	0.0995	49	-0.0223	44	0.0302	56	48.82	36.9
Simulium (Eusimulium) aureum group	0.0235	54	-0.2043	33	-0.0518	63	48.95	37.1
Amphinemura sulcicollis	0.0082	55	0.011	46	0.1746	44	49.51	38.0
Pericoma group	0.1146	48	-0.3764	22	-0.4172	92	49.55	38.1
Dicranota sp.	0.0613	51	-0.0752	40	-0.0098	59	49.61	38.2
Rhithrogena sp.	0.1516	46	-0.0192	44	-0.1144	68	49.97	38.8
Limnius volckmari	0.0127	55	-0.0485	42	0.0727	53	50.05	38.9
Leuctra inermis	0.11	48	0.052	48	0.0226	57	50.09	39.0
Baetis rhodani	0.1359	47	0.0533	48	-0.0466	62	50.61	39.9
Tanypodinae [sub-family]	0.0794	50	0.0842	50	0.0681	53	50.79	40.1
Potamophylax sp.	-0.0492	59	-0.0609	41	0.1157	49	50.83	40.2
Simulium (Simulium) ornatum group	0.0239	54	-0.0353	43	0.0237	57	50.87	40.3
Lumbriculidae	-0.1286	64	-0.1859	34	0.0958	51	51.06	40.6
Esolus parallelepipedus	0.0729	51	0.2126	58	0.2009	42	51.08	40.6
Hydraena sp.	-0.0215	57	-0.0789	40	0.0275	56	51.27	40.9
Elmis aenea	-0.0368	58	-0.0588	41	0.0435	55	51.81	41.8
Ancylus fluviatilis	-0.0668	60	-0.1348	37	0.0091	58	51.82	41.9

TAXON NAME	pCCA axis 1 score	%	pCCA axis 2 score	%	pCCA axis 3 score	%	Weighted Sum of %	MetTol score
Electrogena lateralis	0.2076	42	0.2329	59	-0.0422	62	51.95	42.1
Nemoura avicularis	0.0784	50	-0.1518	36	-0.2544	79	52.03	42.2
Eloeophila sp.	-0.1496	65	-0.1882	34	0.0621	53	52.23	42.5
Ephemera danica	-0.1452	65	-0.1994	33	0.0364	55	52.35	42.7
Polycelis felina	-0.1552	65	-0.1192	38	0.1365	47	52.39	42.8
Isoperla grammatica	-0.0009	55	0.0491	48	0.0402	55	52.93	43.7
Limnephilidae	-0.069	60	-0.1891	34	-0.1134	68	53.04	43.8
Brachyptera risi	-0.1043	62	0.1285	53	0.2741	36	53.26	44.2
Nemoura cambrica group	0.0679	51	-0.0164	44	-0.1748	73	53.53	44.7
Orthocladiinae [sub-family]	-0.0155	56	0.0468	48	0.0226	57	53.63	44.8
Philopotamus montanus	0.0258	54	-0.1891	34	-0.3325	85	54.23	45.8
Tipula (Yamatotipula) montium group	-0.0086	56	-0.0648	41	-0.1545	71	54.44	46.2
Clinocerinae	0.137	47	0.2319	59	-0.0686	64	54.45	46.2
Dinocras cephalotes	-0.0345	58	-0.1864	34	-0.2954	82	55.36	47.7
Odontocerum albicorne	-0.017	56	-0.0712	41	-0.2015	75	55.41	47.7
Wormaldia sp.	-0.1193	63	-0.235	31	-0.2184	76	55.49	47.9
Lumbricidae	-0.1175	63	-0.0996	39	-0.0822	65	55.62	48.1
Tanytarsini [tribe]	-0.0353	58	0.0625	49	-0.0434	62	55.69	48.2
Hydracarina	0.0834	50	0.1967	57	-0.0956	66	55.80	48.4
Asellus aquaticus	0.0186	54	0.1368	53	-0.064	64	55.95	48.6
Pedicia sp.	0.184	44	0.154	54	-0.3131	84	55.97	48.7
Mystacides sp.	-0.2801	73	-0.0499	42	0.2088	42	56.06	48.8
Hydropsyche siltalai	-0.1364	64	0.1479	54	0.1887	43	56.10	48.9
Siphonoperla torrentium	-0.1638	66	0.0185	46	0.0669	53	56.57	49.7
Sericostoma personatum	-0.2456	71	-0.0086	44	0.1426	47	57.05	50.5
Halesus sp.	0.0427	53	0.3224	64	-0.0047	59	57.79	51.7
Perla bipunctata	0.1384	47	0.3928	68	-0.1201	68	58.46	52.8
Ecdyonurus sp.	-0.1701	66	-0.061	41	-0.1762	73	59.58	54.6
Diamesinae [sub-family]	0.2279	41	0.5946	81	-0.1143	68	59.69	54.8
Elodes sp.	-0.1452	65	-0.0124	44	-0.1878	74	60.02	55.3
Naididae	-0.0501	59	0.0744	49	-0.2517	79	60.10	55.5
Chironomini [tribe]	-0.0726	60	0.2157	58	-0.1111	67	60.97	56.9
Oreodytes sanmarkii	-0.1331	64	0.0559	48	-0.2144	76	61.47	57.7
Alainites muticus	-0.1037	62	0.1655	55	-0.1525	71	61.64	58.0
Silo pallipes	-0.3328	77	0.0427	47	0.081	52	61.68	58.1
Lepidostomatidae	-0.3931	81	0.0412	47	0.1599	46	61.98	58.6
Enchytraeidae	-0.434	83	-0.1507	36	-0.0581	63	63.36	60.8
Nematoda	0.1245	47	0.3776	68	-0.407	91	63.73	61.4
Potamopyrgus antipodarum	-0.2606	72	0.0404	47	-0.1765	73	64.18	62.2
Glossosoma sp.	0.0545	52	0.4419	71	-0.2653	80	64.46	62.6
Drusinae	-0.2447	71	0.2105	58	-0.1161	68	65.94	65.1
Gammarus pulex	-0.4307	83	-0.1363	37	-0.2189	76	66.44	65.9
Centroptilum luteolum	-0.1107	62	0.9182	100	0.2696	37	68.88	69.9
Tubificidae	-0.4366	83	0.1759	55	-0.0897	66	70.35	72.3
Paraleptophlebia sp.	-0.6503	97	-0.2479	30	-0.5153	100	75.96	81.6
Ithytrichia sp.	-0.586	93	0.3604	67	-0.0778	65	78.05	85.0
Caenis rivulorum	-0.6977	100	0.6036	81	-0.1426	70	87.18	100.0

B MetTol index testing

Having ranked macroinvertebrate taxa according to their association with metal gradients and assigned tolerance scores (MetTol) we can now calculate a MetTol index value for any river site where the macroinvertebrate community has been similarly sampled and processed by simply averaging the MetTol scores for those taxa present in the sample. We tested the performance of this new biotic index using both the calibration dataset from which it was developed and also an independent dataset drawn from existing datasets.

Calibration dataset testing

The calibration dataset consisted of the 86 sites for which we had direct measures of the metal concentrations in Baetis tissue (Table 4.12). To represent the metal stress gradient in testing we presented the *Baetis* tissue metal concentrations relative to a calculated threshold value above which toxic effects are apparent. For each metal this threshold value was calculated using the same quantile regression approach as described in the analysis of the GBASE sediment chemistry data (Section 4.3.6). Here we related biomonitor tissue metal concentration and macroinvertebrate community taxon richness using quantile regression (95 percentile) to identify the value above which the biomonitor tissue metal concentration constrains the upper range of taxon richness, i.e. the threshold above which toxic effects are apparent. This analysis was carried out for arsenic, cadmium, copper, nickel, lead and zinc against *Baetis*, *Hydropsyche*, *Leuctra* and *Gammarus* tissue metal concentrations. See Section 3.1 for details of quantile regression methods and Section 4.3.6 for results.

Optimal *Baetis* tissue concentration threshold models were identified for three of the six metals (Table 4.17). For lead, the *Baetis* tissue concentration threshold model was close to optimal. For nickel, we applied the average of the values derived from optimal *Hydropsyche* and *Leuctra* tissue concentration threshold models. For zinc we applied the optimal *Leuctra* tissue concentration thresholds.

Table 4.17. Modelled threshold tissue metal concentrations (log μg g^{-1}) for each biomonitor taxa. Values in red are thresholds from close to optimal models. Empty cells indicate where threshold models could not be fitted to the data.

log μg g^{-1}	*Baetis*	*Hydropsyche*	*Leuctra*	*Rhyacophila*
As	2.44	-	2.32	-
Cd	0.94	-	0.89	-
Cu	1.95	1.46	1.90	-
Ni	-	1.93	1.91	-
Pb	0.93	1.38	0.26	-
Zn	-	-	2.47	-

Mean log *Baetis* tissue metal concentrations at each site were divided by the log threshold values to derive a standardised measure of metal stress whereby values greater than unity indicated tissue concentrations above the threshold for community effects. We then related variation in the MetTol index across the 99 calibration sites to the sum of standardised metal concentrations (Sum SMC) and to the maximum standardised metal concentration (Max

SMC). The former measure assumes an additive effect for metal mixtures, while the latter focusses on the metal that is most substantially exceeding its threshold and hence is likely to be the primary stressor at the site. We found a significant negative relationship between MetTol and both measures of metal stress, though the relationship was more pronounced with the maximum standardised metal concentration (Figure 4.11).

We also related other routinely used biotic indices (WHPT-ASPT, WHPT-NTAXA and AWICsp) to the two measures of metal stress and to MetTol to assess whether it offered additional explanatory power over the *status quo*. WHPT-ASPT is a biotic index designed to assess the impacts of organic pollution on stream macroinvertebrates (Walley and Hawkes 1996, Clarke et al., 2011). WHPT-NTAXA is simply the count of WHPT-scoring taxa present in a sample and provides an indication of general degradation. WHPT-ASPT and WHPT-NTAXA are the EU Water Framework Directive-compliant indices used by UK environment agencies to formally report stream water quality at a national scale. AWICsp is a biotic index designed to detect the impact of acidic conditions on stream macroinvertebrates (Murphy et al., 2013). We found that MetTol was consistently better related to metal stress than the other indices (Table 4.18). MetTol was not correlated with WHPT-ASPT but was positively related with WHPT-NTAXA and AWICsp (Table 4.18).

These findings offer encouragement that the MetTol index can used to detect where metal-contaminated sediments are causing ecological impacts. However the index first needs to be tested against an appropriate independent test dataset, as described in Section 4.3.9.

Table 4.18. Pearson correlation coefficients, based on the calibration dataset, between the MetTol index, three other established indices and two measures of metal stress; the sum of standardised metal concentrations (Sum SMC) and the maximum standardised metal concentration (Max SMC). Statistically significant correlations are in bold.

	Sum SMC	Max SMC	WHPT-ASPT	WHPT-NTAXA	AWICsp
MetTol	**-0.531**	**-0.615**	-0.109	**0.312**	**0.278**
WHPT-ASPT	-0.168	0.187	-		-
WHPT-NTAXA	0.021	-0.182	-	-	-
AWICsp	-0.077	-0.126	-	-	-

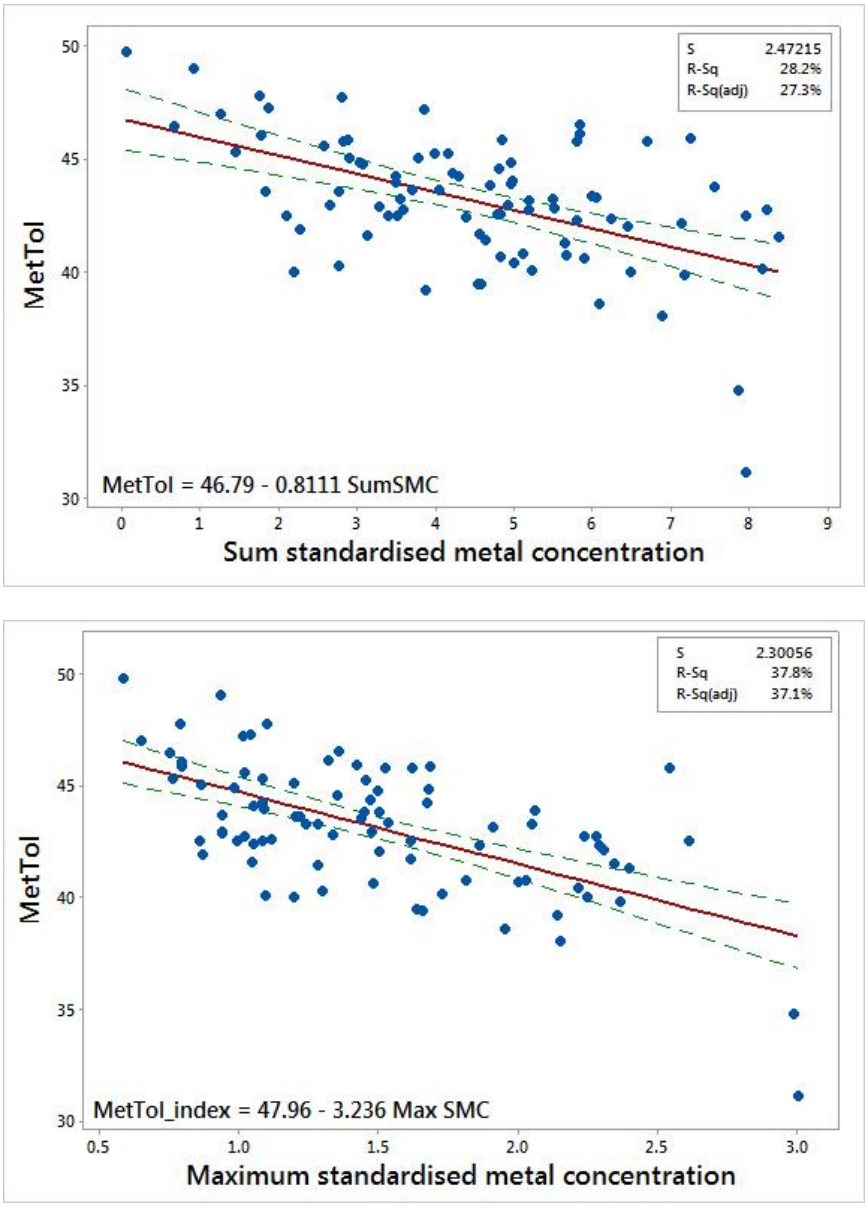


Figure 4.11 Relationship between MetTol index and two measures of metal stress; the sum of standardised metal concentrations (Sum SMC) and the maximum standardised metal concentration (Max SMC) assessed on the calibration dataset.

4.3.8 Independent testing of index

Objective 4b [Test the performance of the new diagnostic index on an independent dataset to confirm its sensitivity to variation in extent of mining-derived metal bioavailability].

To confirm the sensitivity of the new diagnostic index (MetTol) to variation in the extent of mining-derived bioavailable metal [Objective 4b] we tested it on an independent dataset we compiled from existing biological and chemical data sources. We were aware of only two independent UK datasets consisting of macroinvertebrate community data and site-matched metal body burden data. In the first of these, Rainbow et al. (2012) and Awrahman et al. (2016) measured the metal content of specimens of *Hydropsyche*, *Diplectrona* and *Plectrocnemia* caddis flies collected from 30 Cornish stream sites. At each site they also sampled the mayfly community but not the entire macroinvertebrate community, meaning we were not able to use these data to test the MetTol index. We spatially matched 17 of the 30 sites to Environment Agency monitoring sites but the EA macroinvertebrate community data was not resolved to a sufficient taxonomic level to allow calculation of MetTol index values.

A second dataset was collected as part of a study of new approaches to setting environmental quality standards (EQS) for trace metals in the aquatic environment (Bass et al., 2008). This study attempted to establish dose-response relationships for macroinvertebrates and diatoms in stream waters contaminated to different extents with trace metals (cadmium, copper, nickel, lead and zinc). As part of the work, the macroinvertebrate community was sampled at 33 stream sites in Cumbria and the North Pennines using the same standard RIVPACS protocol as was used in the present study (see Section 4.2.1). The 33 sites were purposely selected to span a range of contamination from abandoned metal mines (mainly copper, lead and zinc mines). At each site metal body burdens were estimated for four taxa (*Rhithrogena*, *Leuctra*, Perlodidae and Simuliidae: De Jonge et al., 2013).

We requested and were kindly given access to the macroinvertebrate community, associated water chemistry and metal body burden data from the Bass et al. (2008) study. Macroinvertebrate communities had been sampled and processed appropriately to allow calculation of a MetTol index value. Water chemistry (major solutes, pH and trace metals) was measured at each site on four occasions over a two-month period prior to the macroinvertebrate sampling. Mean trace metal concentrations were calculated from the four sampling occasions. Using the UKTAG metal bioavailability tool (M-BAT) we were able to produce a site-specific estimate of the bioavailable concentrations of copper, nickel, lead and zinc in the stream water. M-BAT is a simplified version of the biotic ligand model (Environment Agency, 2009, WFD-UKTAG, 2014). The bioavailable concentration of each metal was compared with the EQS for the bioavailable fraction and expressed as a risk characterisation ration (RCR). RCR values greater than unity indicate exceedance of targets. RCR values were summed across the four trace metals to derive a combined measure of metal stress. The maximum RCR was also recorded as it indicates the metal that is most substantially exceeding its threshold and hence is likely to be the primary stressor at the site. Macroinvertebrate specimens for metal body burden analysis were collected from each stream site on the same days as the community sampling, returned to the laboratory, processed and analysed using a ICP-MS following the same protocols as in the current study (see Section 4.2.2 and De Jonge et al., 2013). Tissue concentrations of

cadmium, copper, nickel, lead and zinc (log μg g^{-1} dw) in the four biomonitor taxa were related to MetTol index values. Additionally, we related MetTol index values to a measure of combined metal stress by standardising the metal tissue concentrations in *Leuctra* by the threshold values derived using quantile regression in Section 4.3.6. For each site mean measured cadmium, copper and zinc *Leuctra* tissue concentrations were divided by the respective threshold values (Cd:7.7 μg g^{-1}, Cu: 80 μg g^{-1}, Ni: 80 μg g^{-1}, Zn 294 μg g^{-1}). We were not able to determine a threshold value for lead in *Leuctra* so this metal was not included in the measure of combined metal stress. However, De Jonge et al. (2013) considered that cadmium, copper and zinc were the principle metals affecting the macroinvertebrate community at these sites. Standardised metal concentration (SMC) values greater than unity indicate exceedance of biological effect thresholds. Nickel SMC values were considerably below unity at all sites (0.026-0.317, mean = 0.089). SMC values were summed across cadmium, copper, nickel and zinc for each site to provide a measure of combined metal stress. The maximum of the four SMC values was also noted as it indicates the metal that is most substantially exceeding its threshold and, hence, likely to be the primary stressor at the site.

We also collated sediment chemistry data from the British Geological Survey GBASE sampling programme (see Section 2.1.1) for 22 sites that could be spatially matched to Bass et al. (2008) sites. Stream sediments were sampled at these sites between 1985 and 1988, while the macroinvertebrate community was sampled in 2006. Sediment metal concentrations (cadmium, copper, nickel, lead, tin and zinc) were presented relative to macroinvertebrate threshold values derived using quantile regression in Section 3 of the current study (see Table 3.1), i.e. as standardised metal concentration (SMC). As before, SMC values greater than unity indicate exceedance of biological effect thresholds.

Results

There were no significant correlations between MetTol values and variation in summed or maximum RCR values (Figure 4.12), though both correlations were close to significance. Correlations became significant when three outlier sites (Uldale Beck, Dale Head Gill and Mosedale Beck) were excluded from the independent test dataset (Figure 4.13). These sites returned low RCR values and had no mining influences in their catchments, but the invertebrate community indicated significant acidification stress (mean pH 5.2-5.7; AWICsp 3.4-4.3). These three sites were excluded from all subsequent analyses.

When we related MetTol to the predicted bioavailable stream water concentrations for individual metals we found significant negative correlations with copper, lead and zinc, but not nickel (Figure 4.14). However, only two of the sites (Threlkeld and Nent) were markedly affected by high nickel bioavailability in the stream water. Two outlier sites (Dell Beck and Levers Water Beck) in the lead and zinc relationships flowed through Coniston Copper Mine and had among the highest concentrations of bioavailable copper in the dataset; hence their low MetTol scores were most likely as a result of copper impacts.

We found no significant correlation between MetTol and the summed or maximum standardized sediment metal concentrations; though both correlations were significant at $P<0.07$ (Figure 4.15). There were significant negative correlations between MetTol and the standardized sediment concentrations of cadmium, copper, tin and zinc but not for nickel or lead (Figure 4.16). Copper, lead and zinc were the metals most frequently with sediment

SMC values in excess of unity and lead was the metal with maximum sediment SMC value at all sites. Copper was the next most prominent metal in the sediment at Dell Beck and Levers Water Beck; at all other sites it was zinc. The four sites with highest sediment metal content did not have the lowest MetTol values. Two of these four sites (Slei Gill and Black Mires Gill) are in the lead mining area of Swaledale on alkaline geologies, conditions which will have reduced the toxic effects of the high sediment metal levels on the macroinvertebrate fauna (See Section 4.3.4d). Metal-sensitive taxa such as *Gammarus pulex*, *Glossosoma*, *Silo pallipes* and *Alainites muticus* were found at both sites. The other two sites (Roughton Gill and Wood Head Stream) are on the northern edge of the Lake District in a copper and lead mining area and on mixed igneous and sedimentary geology but still support metal-sensitive taxa such as *Gammarus pulex*, Drusinae, *Ecdyonurus,* and *Alainites muticus.* Risk characterization ratios (RCR) of lead and zinc (but not copper) bioavailability from the stream water were relatively high at these sites, in particular at the Lake District streams. In addition, copper, lead and zinc tissue concentrations in biomonitor taxa from these four streams were consistently among the highest in the dataset. Therefore, despite the considerable amount of metals bound to the fine sediment and bioavailable from the water column and the fact that much of these metals are being taken up into tissues, diversity and mean metal-tolerance level of the macroinvertebrate assemblages suggest that the stream faunas were only moderately impacted.

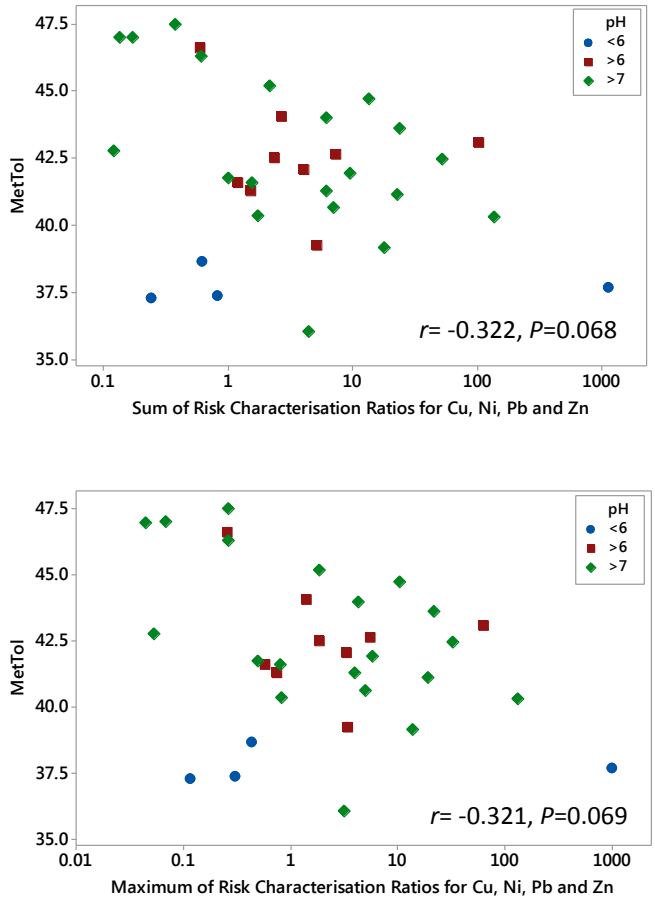


Figure 4.12 Relationship between MetTol index and two measures of bioavailable metal in stream water; the sum of M-BAT risk characterisation ratios and the maximum of M-BAT risk characterisation ratio for copper, nickel, lead and zinc,

assessed on an independent dataset. Mean stream water pH category is indicated by the different symbols. Significance of correlation coefficients (*r*) is indicated within each panel.

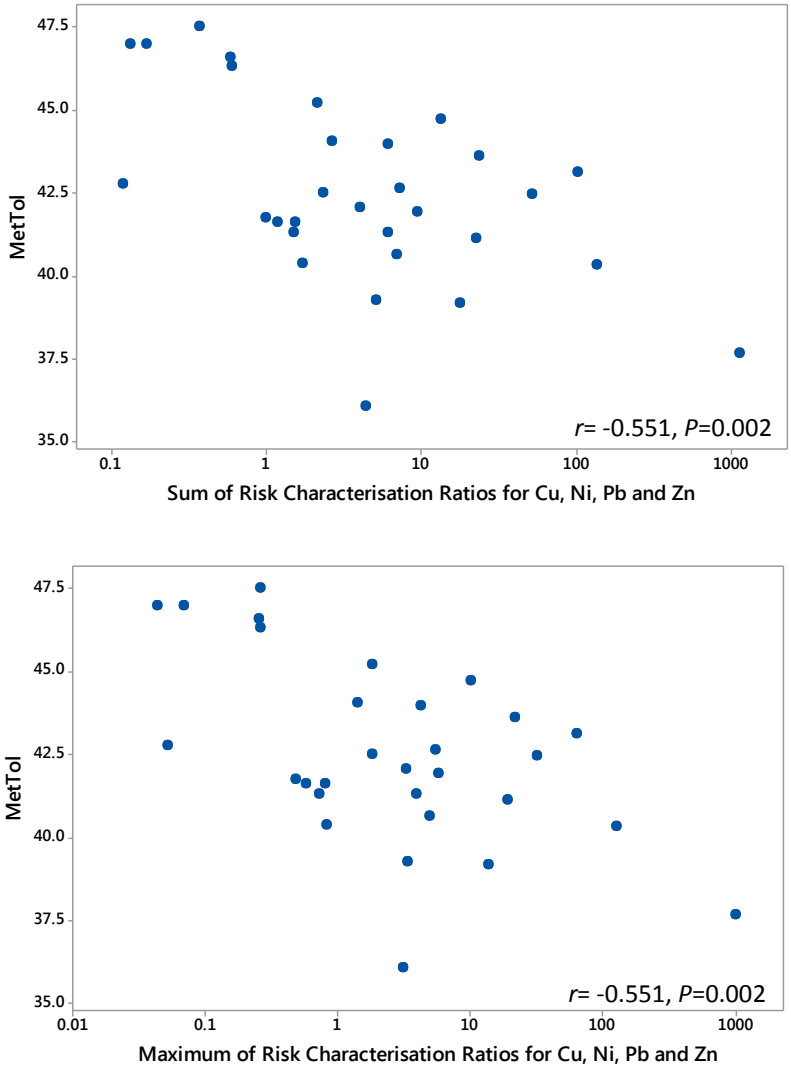


Figure 4.13 Relationship between MetTol index and two measures of bioavailable metal in stream water; the sum of M-BAT risk characterisation ratios and the maximum of M-BAT risk characterisation ratio for copper, nickel, lead and zinc, assessed on an independent dataset, having excluded three non-mining sites impacted by acidification. Significance of correlation coefficients (*r*) is indicated within each panel.

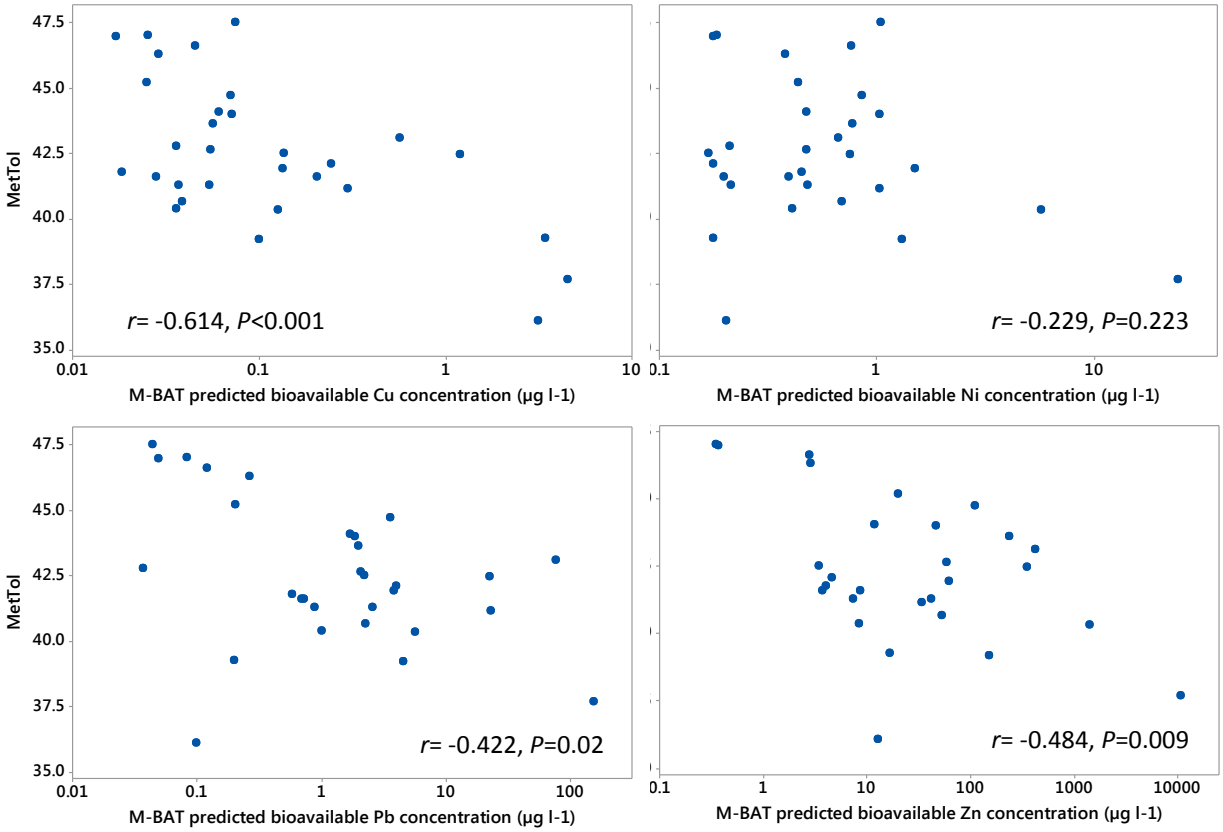


Figure 4.14 Relationship between MetTol index and M-BAT predicted bioavailable stream water concentrations for copper, nickel, lead and zinc, assessed on an independent dataset. Significance of correlation coefficients (*r*) is indicated within each panel.

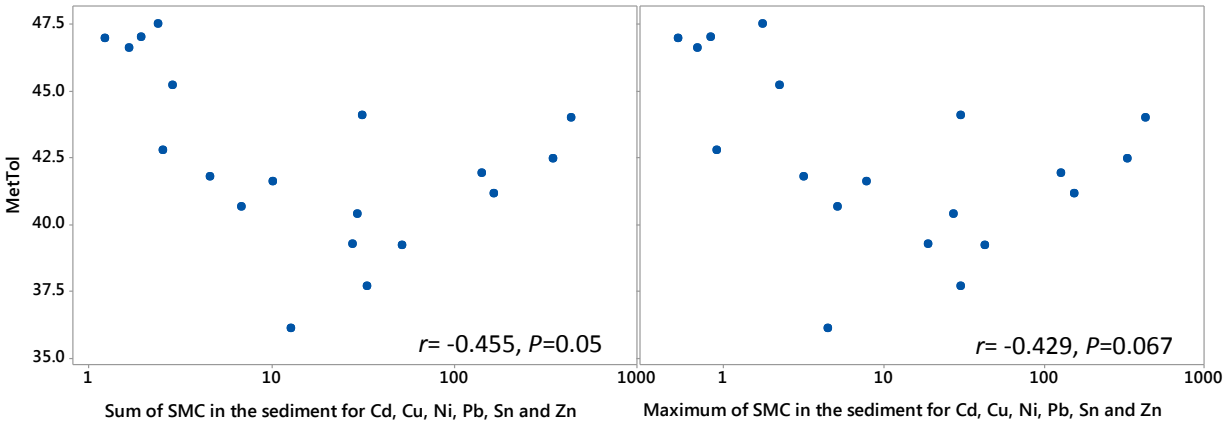


Figure 4.15 Relationship between MetTol index and sum and maximum of standardised sediment metal concentrations (SMC) for cadmium, copper, nickel, lead, tin and zinc. Significance of correlation coefficients (*r*) is indicated within each panel.

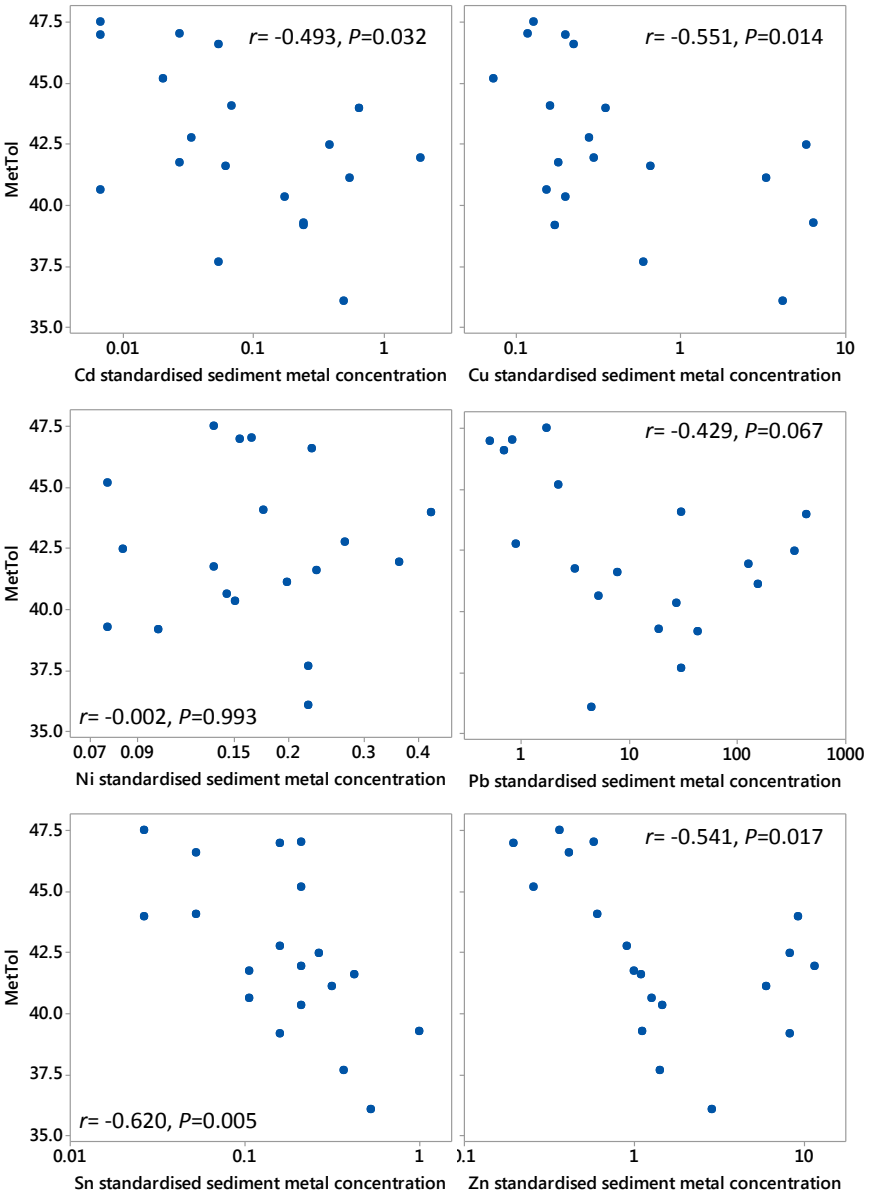


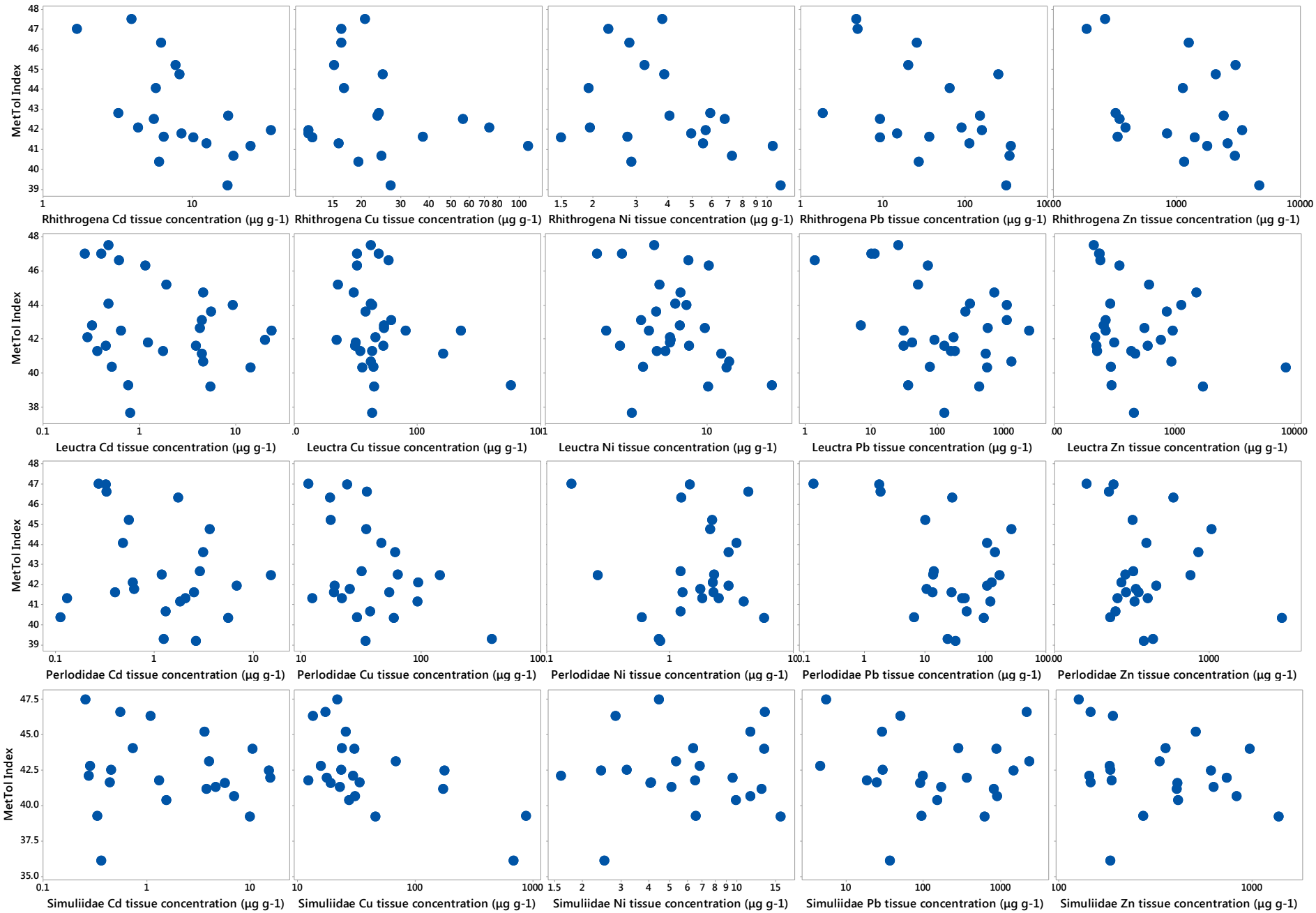
Figure 4.16 Relationship between MetTol index and standardised sediment metal concentrations (SMC) for cadmium, copper, nickel, lead, tin and zinc. Significance of correlation coefficients (*r*) is indicated within each panel.

We found significant negative relationships between the MetTol index and tissue concentrations of cadmium and lead in *Rhithrogena,* lead in *Leuctra*, copper and lead in Perlodidae, and copper in Simuliidae (Table 4.19, Figure 4.17). MetTol values were not correlated with nickel or zinc tissue concentrations in any of the four biomonitor taxa used by De Jonge et al. (2013). As with the MetTol-bioavailable metal relationships, there was considerable scatter of MetTol values for a given metal tissue concentration. This is in part due to the confounding effect of other metals on the bivariate associations between MetTol and a single metal. When we related MetTol to a measure of combined metal stress; SMC based on cadmium, copper, nickel and zinc tissue concentrations in *Leuctra*, we found significant negative relationships with both the sum of SMC (r =-0.396, P=0.033) and the maximum SMC value (r =-0.402, P=0.030: Figure 4.18). At 10 of the 29 sites in the relationship, none of the metals exceeded the biological effects threshold. At four of the sites copper was the metal with the highest SMC values and at the remaining 16 sites zinc was the metal with the highest SMC values.

Table 4.19. Pearson correlations between MetTol index and log-transformed tissue concentrations (log µg g^{-1} dw) of cadmium, copper, nickel, lead and zinc in *Rhithrogena, Leuctra*, Perlodidae, and Simuliidae. Number in parenthesis is the number of sites from which specimens were collected. Significant correlations are indicated in bold; * *P*<0.05, ** *P*<0.01.

	Cd	Cu	Ni	Pb	Zn
Rhithrogena (n=19)	**-0.594****	-0.247	-0.427	**-0.484***	-0.433
Leuctra (n=29)	-0.238	-0.276	-0.315	**-0.399***	-0.298
Perlodidae (n=24)	-0.232	**-0.413***	-0.060	**-0.469***	-0.172
Simuliidae (n=22)	-0.100	**-0.605****	0.052	-0.066	-0.264

Figure 4.17 (overleaf) Relationships between MetTol index and tissue concentrations (µg g^{-1} dw) of cadmium, copper, nickel, lead and zinc in *Rhithrogena, Leuctra*, Perlodidae, and Simuliidae. See Table 4.19 for details of statistical significance of relationships.


MetTol Index
Rhithrogena Cd tissue concentration (µg g-1)
Rhithrogena Cu tissue concentration (µg g-1)
Rhithrogena Ni tissue concentration (µg g-1)
Rhithrogena Pb tissue concentration (µg g-1)
Rhithrogena Zn tissue concentration (µg g-1)
Leuctra Cd tissue concentration (µg g-1)
Leuctra Cu tissue concentration (µg g-1)
Leuctra Ni tissue concentration (µg g-1)
Leuctra Pb tissue concentration (µg g-1)
Leuctra Zn tissue concentration (µg g-1)
Perlodidae Cd tissue concentration (µg g-1)
Perlodidae Cu tissue concentration (µg g-1)
Perlodidae Ni tissue concentration (µg g-1)
Perlodidae Pb tissue concentration (µg g-1)
Perlodidae Zn tissue concentration (µg g-1)
Simuliidae Cd tissue concentration (µg g-1)
Simuliidae Cu tissue concentration (µg g-1)
Simuliidae Ni tissue concentration (µg g-1)
Simuliidae Pb tissue concentration (µg g-1)
Simuliidae Zn tissue concentration (µg g-1)

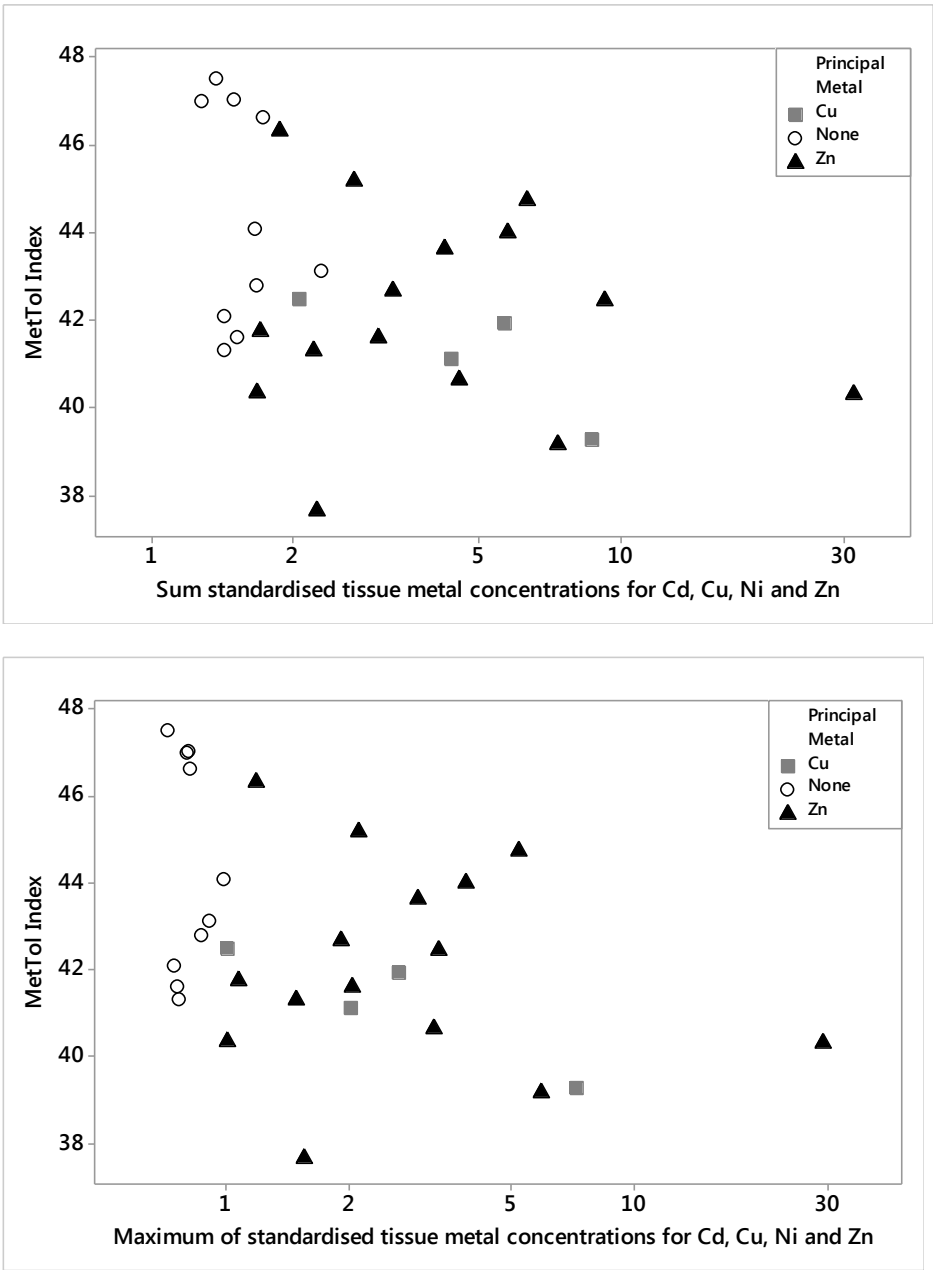


Figure 4.18 Relationship between MetTol index and sum and maximum of standardised tissue metal concentrations (SMC) for cadmium, copper, nickel and zinc in *Leuctra*. Symbols indicate the metal with the highest SMC value in exceedance of the biological effects threshold at each site.

Finally, we compared the relationship between concentrations of each of cadmium, copper, nickel, lead and zinc in the tissues of *Leuctra* and metal concentrations in the sediments and bioavailable concentrations in the stream water to give some insight into which uptake route, sediment or water, was the more important. Bioavailable concentrations of cadmium in stream water were not available so we used the stream water concentrations instead for this metal. We found significant positive correlations between cadmium, copper, lead and zinc tissue concentrations and both sediment and stream water concentrations (Figure 4.19). However, for copper, lead and zinc the relationships with sediment metal concentrations were more pronounced than those with bioavailable stream water concentrations.

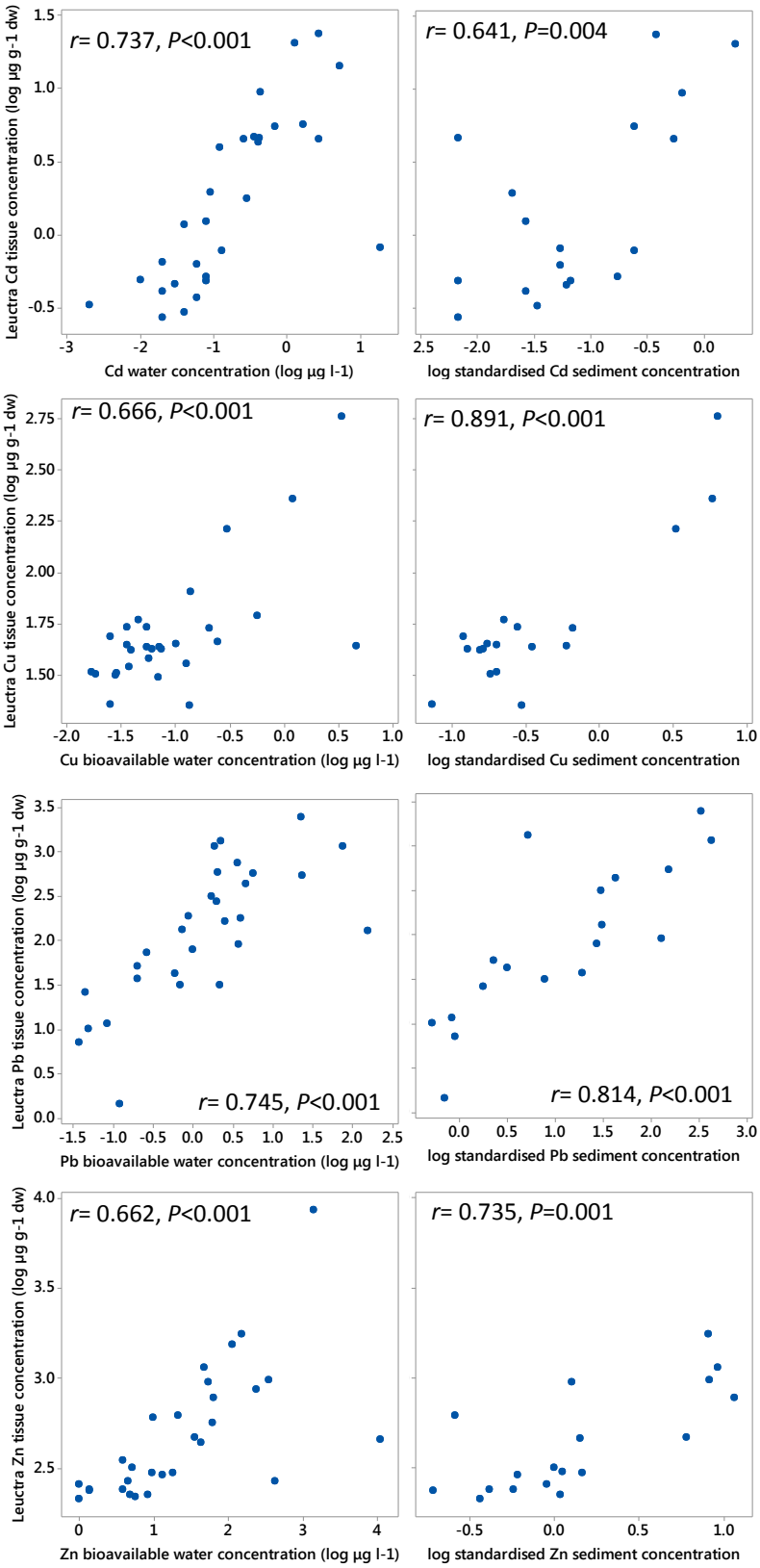


Figure 4.19 Relationship between concentrations of each of cadmium, copper, lead and zinc in the tissues of *Leuctra* and standardised metal concentrations in the sediments and bioavailable concentrations in the stream water (stream water concentration for cadmium). Significance of correlation coefficients (*r*) is indicated within each panel.

There were no significant relationships between nickel tissue concentrations and the bioavailable fraction in stream water or nickel in the sediment (r_{bio} = 0.102, p = 0.60; r_{sed} = -0.095, p = 0.71).

Overall, the independent testing has confirmed that the new MetTol index is sensitive to bioavailable metal contamination, be it quantified from analysis of tissue concentrations in selected biomonitor taxa, predicted from stream water chemistry or inferred from metal concentrations in the bed sediment. In this test dataset, variation in MetTol values were strongly associated with bioavailable copper in stream water, overall metal bioavailability in stream water (sum and maximum RCR), copper concentrations in Simuliidae tissues, cadmium concentrations in *Rhithrogena* tissues, and copper, nickel and zinc concentrations in stream sediments.

5 Dose-Response Curves

Objective 3b

Derive dose–response curves for metal effects in streams and estuaries using the combination of bioaccumulated metal data and traditional measures of stress, such as changes in community structure.

5.1 Methods

Two approaches were taken to identify the threshold bed sediment concentrations beyond which river macroinvertebrate communities are likely to be harmed by a given metal. First we used the approach to deriving ecological effect concentrations described by de Deckere et al. (2011) to calculate the Lowest Effect Level (LEL) and the Severe Effect Level (SEL). Second, we used species sensitivity distributions (SSD) to identify the Hazardous Concentration (HC) to 5%, 10% and 20% of taxa (HC_5, HC_{10}, HC_{20}). The SSD approach involves plotting the cumulative distribution of sensitivities of taxa against a threshold concentration, e.g. 50 or 90 percentile of the distribution of measured bed sediment concentrations at sites where the taxon is present. From the SSD, a lower limit is estimated determined by the proportion of the taxa used in the analysis likely to be impacted if sediment concentrations exceed this value, which then should be protective for the majority of the taxa assessed (Posthuma et al., 2002).

5.1.1 Sediment Effect Concentrations

Taxa were selected from the field survey dataset (90 most frequently occurring taxa across 99 sites) for inclusion in species sensitivity distributions according to their tolerance to each of the five main metals of concern, cadmium, copper, nickel, lead and zinc. For each metal, 24 taxa were selected to represent the full range of responses evident from the partial ordinations carried out as part of the MetTol index development, from the most sensitive taxon to the taxon that was found to be most tolerant of each metal. For each taxon, the distribution of bed sediment metal concentrations (log mg kg^{-1}) across sites where the taxon was present was summarised as a cumulative percentage histogram (Figure 5.1), from which the 50^{th} and 90^{th} percentile bed sediment metal concentrations were derived, i.e. the metal concentration below which 50% and 90% of occurrences of that taxon were recorded. The 50^{th} percentile value represents an estimate of the mid-point of the distribution of each taxon, in terms of the response to sediment metal concentrations, whereas the 90^{th} percentile represents an estimate of the upper limit of the distribution of each taxa. Estimates of the 50^{th} percentile are more robust than corresponding estimates of the 90^{th} percentile, but in terms of protecting species from hazard, the 90^{th} percentile is probably more relevant.

The 5^{th} percentile and 95^{th} percentile of the distribution of 90^{th} percentile bed sediment metal concentrations for the 24 selected taxa were then derived, which represent the LEL and SEL respectively. These values were compared with those calculated by de Deckere et al. (2011) based on benthic macroinvertebrate data from 600 freshwater sites in Flanders, northern Belgium.

5.1.2 Species Sensitivity Distributions

SSDs were generated for each metal by plotting the cumulative distribution of the 90th percentile values of the 24 taxa (Figure 5.2), then modelling the variation in sensitivity across the sediment concentration gradient and estimating the 5, 10 and 20 percentiles of the distribution based on the chosen model. The 5, 10 and 20 percentiles of the distribution based on the chosen model correspond to the sediment metal concentrations that present a hazard to 5, 10, and 20% of taxa. SSDs were also created based on the cumulative distribution of the 50th percentile values of the 24 taxa.

5.2 Results

With the exception of the SEL for cadmium, the sediment metal concentrations corresponding to the Lowest Effect Level (LEL) and the Severe Effect Level (SEL) calculated using the approach of de Deckere et al. (2011) were higher than the corresponding concentrations derived from the Flemish freshwater sites (Table 5.1). This may be a consequence of the current study targeting catchments containing abandoned metal mining facilities and, thus, using a wider range of sediment metal concentrations, rather than a true biological difference between the two areas of study.

Table 5.1 Lowest effect level (LEL) and Severe effect level (SEL) values (mg kg^{-1}) for cadmium, copper, nickel, lead and zinc derived from the current study dataset and from the de Deckere et al. (2011) study of Flemish freshwater sites.

	Current study		De Deckere et al. (2011)	
	LEL (5 percentile)	SEL (95 percentile)	LEL (5 percentile)	SEL (95 percentile)
Cd	4.7	11.0	0.71	13
Cu	37.9	225	13	85
Ni	39.8	55.7	15	44
Pb	133	3108	19	167
Zn	447	1413	129	1300

For most metals the SSD was best explained by a probit rather than a linear relationship, suggesting a few sensitive and/or tolerant species. As the uncertainty associated with fitting the 50th percentile of species occurrences is lower than that associated with fitting the 90th percentile, the prediction limits for hazardous concentrations (HC_x) derived from the former are smaller than those derived from the latter (Table 5.2). Although hazardous concentrations (HC_5, HC_{10}, HC_{20}) were derived from both, those based on the sediment metal concentration below which 90% of occurrences of that taxon were recorded are probably more relevant in terms of protecting species, as this represents an upper limit of sediment metal concentrations which the species can tolerate (Table 5.2).

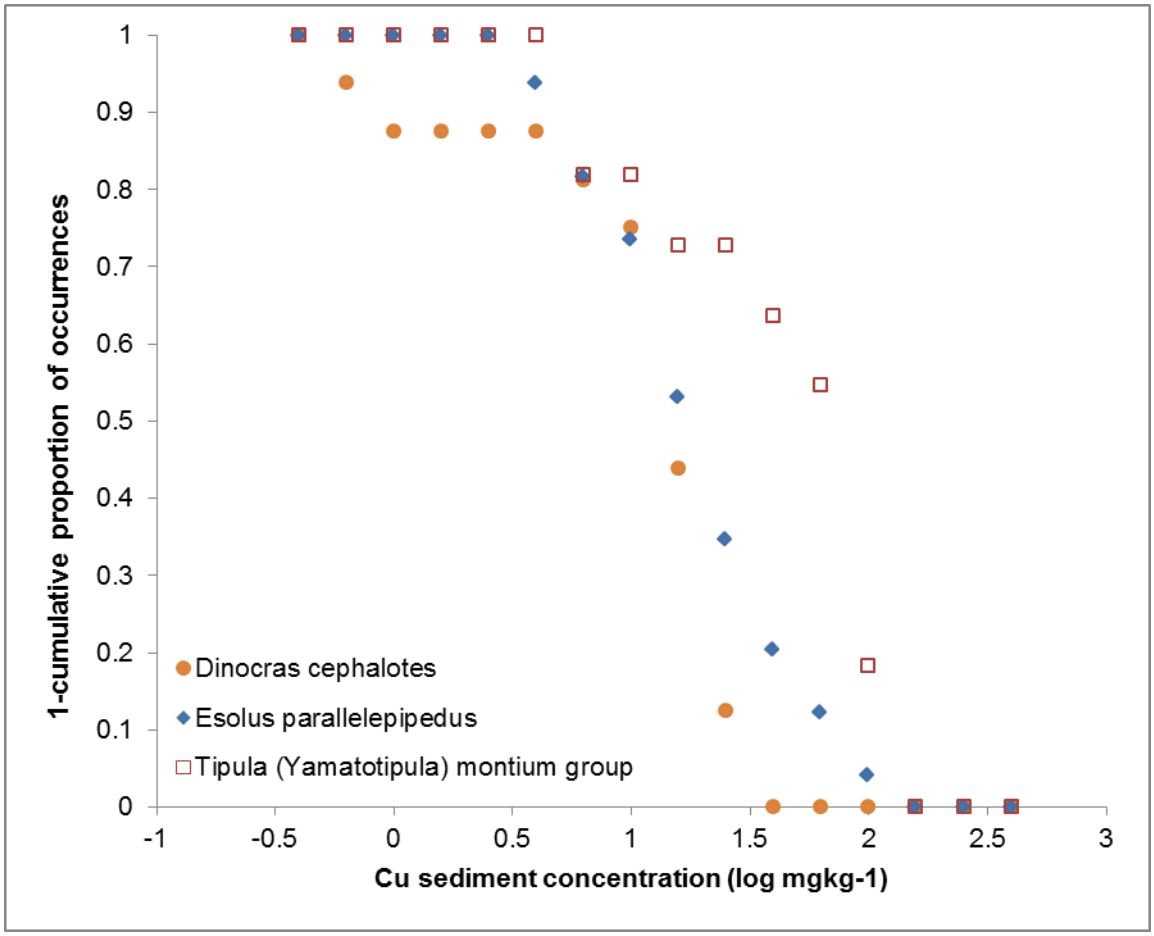


Figure 5.1 Cumulative percentage histogram of taxon occurrences across the copper sediment concentration gradient (log mgkg^{-1}) for three example taxa; *Dinocras cephalotes*, *Esolus parallelepipedus* and *Tipula montium* group.

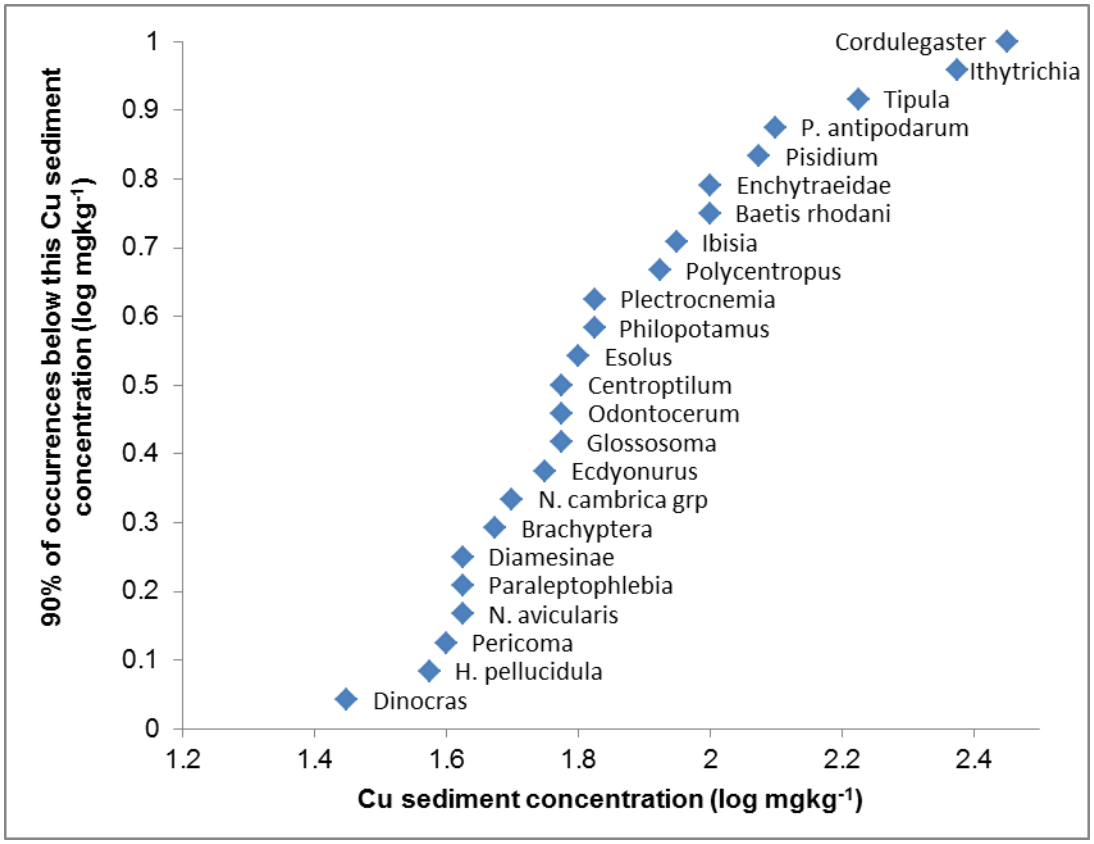


Figure 5.2 Copper sediment concentrations (log mgkg^{-1}) below which 90% of taxon occurrences are recorded for 24 taxa selected to represent the full range of responses evident in the dataset.

Table 5.2 Hazardous concentrations to 5%, 10% and 20% (HC_5, HC_{10}, HC_{20}) of taxa assessed with associated prediction limits based on 50th percentile and the 90th percentile of the distribution of measured bed sediment metal concentrations (mg kg^{-1}) at sites where the taxon was present. The form of the Species Sensitivity Distribution model used to estimate the HC values is also indicated.

		Based on 50th percentile	Lower PI	Upper PI	SSD Model	Based on 90th percentile	Lower PI	Upper PI	SSD Model
Cd	HC_5	2.1	1.9	2.4	Linear	4.7	4.0	5.5	Linear
	HC_{10}	2.2	2.0	2.5		4.9	4.2	5.7	
	HC_{20}	2.3	2.1	2.6		5.3	4.5	6.2	
Cu	HC_5	10.4	9.5	11.3	Linear	27.6	21.5	35.5	Probit
	HC_{10}	10.9	10.0	11.9		34.3	26.8	43.9	
	HC_{20}	12.1	11.1	13.2		44.5	34.9	56.7	
Ni	HC_5	18.2	17.7	18.7	Probit	37.9	36.4	39.5	Probit
	HC_{10}	19.1	18.6	19.7		39.8	38.2	41.4	
	HC_{20}	20.4	19.8	20.9		42.2	40.5	43.9	
Pb	HC_5	21.6	15.6	30.0	Probit	151	99	231	Probit
	HC_{10}	27.8	20.2	38.3		220	145	334	
	HC_{20}	37.7	27.5	51.7		348	231	524	
Zn	HC_5	132	121	144	Probit	431	402	463	Probit
	HC_{10}	143	131	156		498	465	535	
	HC_{20}	157	144	171		594	554	636	

5.3 Comparison among the various approaches used

A number of approaches have been used in this report to establish thresholds for sediment metal concentrations based on ecological data. Each approach has its benefits (e.g. in terms of the range of species and environmental conditions represented in the data used) and drawbacks (e.g. in terms of uncertainties associated with the data). Here we compare the values derived using these various approaches, and existing sediment quality guidelines (Table 5.3).

Broadly, the approaches fall into three classes, a) approaches based on species sensitivities established through ordination of newly collected field data (Section 5), b) thresholds derived from species richness and measured bioavailability (as body burden), in turn translated into a sediment concentration (Section 4.3.6**)**, and c) thresholds based on relationships between G-BASE sediment metal concentrations and EA/NRW biological monitoring data (Section 3).

Both the Canadian and Australasian sediment quality guidelines (SQG) are based on laboratory-based toxicological studies. The Canadian interim sediment quality guidelines are based on the upper limit of the range of sediment chemical concentrations that is dominated by no-effect data entries (corresponding to a threshold effect level; the geometric mean of the lower 15th percentile concentration of the effect data set), such that adverse biological effects are not predicted when the measured concentrations are at or below guideline concentrations (CCME, 1995). The ANZECC and ARMCANZ low trigger value corresponds to a 10% statistical probability of effects when tested against only one or two species, principally amphipods (Long et al. 1995).

Lowest Effect Levels (LEL) calculated using the approach of de Deckere et al. (2011) approximated to the Hazardous Concentrations (HC_5, HC_{10}, HC_{20}) determined from species sensitivity distributions (SSD)**,** as both are derived from the same data. The approach based on quantile regression using biomonitor body burdens returned considerably higher threshold concentrations for cadmium, nickel and zinc than the other approaches. For cadmium and nickel the relationships between body burden and sediment metal concentrations were weak (Figure 4.5), which introduces considerable uncertainty into the values derived. Even for the other metals derived using this approach, the two-step calculation (derivation of threshold/translation into sediment concentration) increases the uncertainty of the thresholds.

With the exception of copper, and to an extent lead, all thresholds derived using ecological data were considerably higher than existing Canadian and Australasian guidlines based on toxicological data (Table 5.3). For copper, the thresholds based on species sensitivities (27.6 – 44.5 mg kg^{-1}) approximate to the Canadian interim sediment quality guidelines (35.7 mg kg^{-1}), whereas those based on quantile regression (75 – 86.2 mg kg^{-1}) approximate to the ANZECC and ARMCANZ low trigger value (65 mg kg^{-1}). For lead, the thresholds based on quantile regression approaches using the body burden of biomonitors (54 mg kg^{-1}) and the lowest BQE metric using G-BASE data (49.6 mg kg^{-1}) approximate to the ANZECC and ARMCANZ low trigger value (50 mg kg^{-1}), whereas the other thresholds based on ecological data were higher (LEL = 151 mg kg^{-1} – HC_{20} = 348 mg kg^{-1}).

Table 5.3 Thresholds for sediment metal concentrations (mg kg^{-1}) based on ecological data.

			Section 5				Section 4.3.6	Section 3		
	CCME°	ANZECC and ARMCANZ*	Sediment Effect Concentrations	Species Sensitivity Distributions Based on 90th percentile			Quantile regression biomonitor body burden	Quantile regression G-BASE data		
	Interim SQG	Low Trigger Value	LEL	HC_5	HC_{10}	HC_{20}	Mean threshold	Geometric mean based on number of taxa	Lowest based on number of taxa	Lowest of all BQE metrics
Ag		1						7.9	7.9	7.9
As	5.9	20					384 [a]	59.6	27	27
Cd	0.6	1.5	4.7	4.7	4.9	5.3	54[b]	6.8	4	3.1
Cu	35.7	65	37.9	27.6	34.3	44.5	75	86.2	75	32.6
Ni		21	39.8	37.9	39.8	42.2	384[b]	109	41.4	41.4
Pb	35	50	133	151	220	348	54	295	49.6	49.6
Sn								17.4	9	9
Sb		2						5	1.8	1.8
Zn	123	200	447	431	498	594	1,561	849	286	153

Thresholds derived using a) species sensitivities used to derive lowest effect levels (De Deckere et al., 2011) and hazardous sediment concentrations to 5%, 10% and 20% (HC_5, HC_{10}, HC_{20}) of taxa, b) sediment metal concentrations and thresholds from quantile regression between biomonitor body burden and species richness, and c) threshold quantile regression using data from G-BASE and EA/NRW biological quality monitoring data. Existing safe limits for North America and Australasia are shown for comparison

° Canadian Council of Ministers of the Environment. 1999. Canadian sediment quality guidelines for the protection of aquatic life: Winnipeg

* ANZECC and ARMCANZ [Australian and New Zealand Environment and Conservation Council, Agriculture and Resource Management Council of Australia and New Zealand] (2000) *Australian and New Zealand guidelines for fresh and marine water quality. Volume 1, The guidelines.* National water quality management strategy; no.4.

[a] Based on relationship with fine sediment <63 µm samples used for source apportionment.

[b] Based on poor relationship between sediment metal concentration and biomonitor body burden

For cadmium, the thresholds based on species sensitivities (4.7 – 5.3 mg kg^{-1}) and on G-BASE quantile regression (3.1 – 6.8 mg kg^{-1}) were approximately an order of magnitude above the Canadian interim sediment quality guidelines (0.6 mg kg^{-1}), and two to four times the ANZECC and ARMCANZ low trigger value (1.5 mg kg^{-1}).

For nickel, the safe limits based on species sensitivities (37.9 – 42.2 mg kg^{-1}) and on the lowest metric in the G-BASE quantile regression were approximately twice the ANZECC and ARMCANZ low trigger value (21 mg kg^{-1}). The other quantile regressions returned concentrations approximately an order of magnitude above the low trigger value.

For zinc, the safe limits based on species sensitivities (431 – 594 mg kg^{-1}) were approximately twice the ANZECC and ARMCANZ low trigger value (200 mg kg^{-1}), and four times the Canadian interim sediment quality guidelines (123 mg kg^{-1}). With the exception of the lowest metric in the G-BASE analysis, safe limits based on quantile regression were higher than those based on species sensitivities.

5.4 Conclusions

The safe limits derived from ecological data most consistent with existing sediment guidelines were for copper.

The safe limits for other metals (cadmium, nickel, lead and zinc) derived from ecological data were approximately two to four times higher than the ANZECC and ARMCANZ low trigger value, and up to an order of magnitude above the Canadian interim sediment quality guidelines. These existing guidelines, based on toxicological data, may be too precautionary, and we suggest that guideline sediment concentrations based on the species sensitivities derived from ecological data may provide a more appropriate level of protection for the environment.

Of the approaches used, the sediment thresholds based on biomonitor body burden were the most uncertain (due to the two-step process used in their calculation). We suggest that these values are best used as confirmation where they are consistent with thresholds derived by other approaches. As sediment metal concentrations were measured with either X Ray Fluorescence Spectroscopy (XRFS) or Direct-reading DC Arc Optical Emission Spectrometry (DCOES), rather than acid extraction, we suggest that less weight is given to the thresholds based on these data where other data are available. We suggest that the thresholds derived from analysis of the field data collected in this project, where the methods for estimating sediment metal concentrations are more consistent with monitoring approaches used, are the most reliable. Here, two approaches were used, to derive Lowest Effect Levels (LEL) and Hazardous Concentrations (HC_5, HC_{10}, HC_{20}). As the HC_5 is towards the lower tail of the species sensitivity distribution (SSD), there will be more uncertainty associated with the derived threshold at this point in the curve compared with HC_{10}, and suggest the latter provides a more robust estimate. For this reason, we suggest that the lowest threshold derived using these two approaches (LEL and HC_{10}) is used, although this deviates from conventional practice when estimating thresholds for an SSD.

Due to the influence of environmental conditions, particularly pH, on bioavailability noted in the field data, we also suggest caution in setting a single threshold for all environmental conditions.

Table 5.3 Summary of thresholds for sediment metal concentrations (mg kg^{-1}) based on ecological data.

	Existing Safe Limits		Based on Ecological Data					Suggested Threshold
	Canada°	Australia and NZ*	SEC†	SSD	Biomonitor body burden	Quantile regression G-BASE data		
	Interim SQG‡	Low Trigger Value	LEL	HC_{10}	Mean threshold	Geometric mean of Nº Taxa	Lowest of Nº taxa	
Ag		1				7.9	7.9	**7.9**
As	5.9	20			384	59.6	27	**27**
Cd	0.6	1.5	4.7	4.9		6.8	4	**4.7**
Cu	35.7	65	37.9	34.3	75	86.2	32.6	**34.3**
Ni		21	39.8	39.8		109	41.4	**39.8**
Pb	35	50	133	220	54	295	49.6	**133**
Sn						17.4	9	**9**
Sb		2				5	1.8	**1.8**
Zn	123	200	447	498	1,561	849	286	**447**

° Canadian Council of Ministers of the Environment (1999)
* Australian and New Zealand Environment and Conservation Council, Agriculture and Resource Management Council of Australia and New Zealand (2000)
† Sediment Effect Concentrations
‡ Sediment Quality Guidelines

6 Source Apportionment

Objective 3b
Source apportionment analysis, carried out on a sub-set of the targeted field survey sites, will be used to quantify the relative importance of different catchment sources of fine sediment and thus provide an insight into how the contamination is being delivered to the point of impact.

As part of the tasks comprising work package 3, source apportionment of fine-grained sediment (< 63 µm) was investigated at the most downstream freshwater depositional site in 20 of the study catchments. Here, the potential fine-grained sediment sources in each catchment, including mine waste material, were characterised and a statistical approach used to determine the relative importance of mine waste as a source of fine sediment to the river.

6.1 Methods

6.1.1 Field sampling of catchment source and sediment samples

A source material sampling exercise was conducted in the 20 catchments where the relevant potential primary fine-grained sediment source types were identified and characterised. These sources were agricultural (grass and arable) topsoils, damaged road verges, channel banks/subsurface sources, urban street dust and the dis-used metal mines. Samples retrieved from agricultural fields and damaged road verges represented the uppermost 0 - 2 cm susceptible to runoff detachment and mobilisation during precipitation events. Care was taken not to sample road sediment deposits, but rather, primary road verge material damaged by passing vehicles or poached by livestock movements. Representative samples of damaged road verges were retrieved from 100 m sections of road margins exhibiting erosion and selected randomly to be representative of the entire road network in each study catchment where this source was included in the sample collection programme. Between 5-10 sub-samples were collected from each 100 m road section targeted by the sampling exercise and bulked into composites. Collection of channel bank samples avoided the uppermost (0-2 cm) topsoil at the river edge and focused on the lower horizons (B and C) exposed by fluvial erosion. These samples included material from the entire bank face comprising the B and C horizons at each channel location, rather than from a specific point within the vertical profile of any given channel bank face. Subsurface sampling targeted features of incision, including any gullies observed on agricultural fields or incised farm tracks, again collecting material below (lower than 2 cm depth) the soil surface. Although the catchments were all largely rural (to avoid any effects of urban development, e.g. sewage treatment works, on ecology) the settlements were present in the catchments, Samples of “urban street dust” were collected using a dustpan and brush and typically involved the retrieval of deposits (sub-samples = ~5 per composite sample) near road drains along the streets of rural settlements (villages and hamlets) within the catchments. Samples of mine waste were primarily collected from slag, spoils heaps and tailings as appropriate, depending on the structure of the mine site and ease of accessibility. The most extensive and actively eroding spoil heaps were found at the study sites in Wales and Northern England. Advice from the Environment Agency was used to help target the most actively eroding spoil heaps in these study regions. Each individual source sample collected from the agricultural topsoil and mine sources represented a composite of sub-samples (n = 5 – 10)

representative of that source across the 100 – 500 m^2 within the immediate vicinity of the sampling point (Table 6.1). In total, 804 composite source material samples were retrieved from the study catchments.

Table 6.1: Summary of the source and channel bed sediment samples collected to characterise key sources of fine-grained sediment identified in the 20 catchments. (A zero indicates that the source type was identified as not important for the catchment in question).

Catchment		Region	Bed Sediment	Channel Bank	Top Soil	Mine Waste	Damaged Roadside Verge	Urban Street Dust
A	St Lawrence Stream	SW	12	9	10	14	9	10
B	Bolingey Stream	SW	12	10	10	18	10	0
C	Porthleven Stream	SW	12	9	9	8	5	5
D	Hayle	SW	12	10	10	12	10	5
E	River Burn (Tavy)	SW	12	9	9	12	10	7
F	Mardle	SW	12	10	10	12	10	0
G	Afon Melindwr	W	12	10	10	5	10	9
H	Nant Magwr	W	12	10	10	12	10	1
I	Afon Cyneiniog	W	12	11	11	11	0	0
J	Afon Ystwyth	W	12	10	10	12	10	0
K	Wye	W	12	10	10	9	0	0
L	Rea Brook	M	12	10	10	9	7	9
M	River South Tyne	NE	12	8	10	12	9	5
N	Red Tarn Beck	NE	12	3	10	11	0	7
O	River Greta	NE	10	9	10	10	2	9
P	Arkle Beck	NE	12	8	10	12	0	0
Q	Egglestone Burn	NE	12	9	9	12	2	0
R	Hudeshope Beck	NE	12	10	10	12	5	7
S	Bedburn Beck	NE	12	8	9	10	4	0
T	River East Allen	NE	12	10	10	12	9	5

Representative channel bed sediment samples were collected from the outlet of the 20 study catchments using a re-suspension method (Lambert and Walling, 1988; Collins and Walling 2007; Duerdoth et al., 2015) in order to assess the primary sources of the local sediment-associated pollution problem. During sample collection, a purpose-built metal stilling well (height 1.1 m, surface area 0.18 m) was carefully lowered onto, and pushed into, the river bed to provide a means of minimising the loss of remobilised sediment by winnowing. The river water and upper 5 cm of the channel bed enclosed in the stilling well were stirred and agitated using a portable battery-powered drill equipped with a plaster stirrer fitting (Collins et al., 2012b). Previous work using a hand held rod has identified the potential problem of inconsistent effort to collect samples on a purely manual basis (Lambert and Walling, 1988; Collins and Walling, 2007). Regular replacement of the drill batteries helped to ensure consistent agitation during the collection of samples. Agitation of both the water column and river bed provided a basis for sampling sediment stored both as a surface drape and within the interstices of the gravel matrix. Each bed sediment sample (total volume of 5 L) comprised a composite of two sub-samples (~ 2.5 L each) retrieved from different points in

the channel at the outlet of each study catchment. A total of 12 (10 in the case of the River Greta) composite samples were retrieved from the catchment outlets (Table 6.1). On this basis, a total of 238 composite channel bed sediment samples were collected for laboratory analyses. The river water and substrate were consistently agitated for 60 seconds prior to the depth-integrated sampling of the remobilised sediment within the stilling well. Collection of sediment to a depth of 5 cm helped to ensure retrieval of interstitial material from the most oxygenated layer of the river bed and thereby the minimisation of issues associated with the potential geochemical transformation of sediment fingerprint properties that could occur deeper in the river substrate where more anoxic conditions might exist (cf. Collins et al., 2012c). The river channel bed at the catchment outlets was sampled on a single basis given the resources available to this project. Single site visits have been used by international studies to characterise the geochemical properties of channel bed sediment in river systems (e.g. Horowitz et al., 2012).

6.1.2 Laboratory work and analyses

All samples retrieved to characterise the source type end members were returned to the laboratory, oven-dried at 40 °C, manually disaggregated using a rubber-tipped pestle and mortar and homogenised using a 63 µm sieve (cf. Collins et al., 1997). Channel bed sediment samples were returned to the laboratory on the day of sampling in acid-washed polyethylene containers, de-watered using settling and decanting, freeze-dried and sieved to <63 µm. Following pre-treatment with hydrogen peroxide at 100 °C on a hotplate to destroy organics, chemical dispersion with calgon and sonification (~ 5 minutes per sample), the ultimate grain size distribution of all catchment source and channel bed sediment samples was measured using a Malvern Mastersizer laser diffraction granulometer. The grain size measurements were used to estimate sample specific surface area as a surrogate for absolute grain size distribution and because this parameter is used in the numerical mass balance modelling for predicting sediment source apportionment. Concentrations of potential geochemical fingerprint properties were determined using a combination of ICP-OES and ICP-MS, following an acid digest using aqua regia. Concentrations of Al, As, Ba, Bi, Cr, Cu, Fe, K, Li, Mg, Mn, Na, Ni, Pb, Sr, Ti and Zn were determined using ICP-OES, whilst the concentrations of Cd, Ce, Co, Cs, Dy, Er, Eu, Ga, Gd, Ge, Hf, Ho, La, Lu, Mo, Nd, Pr, Rb, Sb, Sc, Sm, Sn, Tb, Tl, Tm, U, V, Y, Yb and Zr were measured using ICP-MS.

6.1.3 Statistical verification of source end member discrimination

A data processing procedure outlined in Collins et al. (2012c, 2013, 2014) was modified for the purposes of the work reported here. It was considered important to test for normality prior to proceeding with the selection of scaling metrics for fingerprint property parameter location and scale (Arcones and Wang, 2006). The Lilliefors test (Lilliefors, 1969; Henderson, 2006) was used to assess normality prior to the selection of appropriate metrics for estimating the location (e.g. mean or median) and scale (e.g. deviation) of the source end member and channel bed sediment fingerprint property datasets during the mass balance modelling. As an example, Tables 6.2 and 6.3 show the results of the Lilliefors test for the Afon Cyneiniog and Afon Melindwr study catchments. Appendix A (Tables A1-A18) presents the Lilliefors test results for the remainder of the study catchments. A statistically significant ($p \leq 0.05$) result indicates that the tracer property in question does not conform to a uniform distribution. The results of the Lilliefors tests confirmed that not all tracers were characterised by uniform distributions. On the basis of these results, the measured median

and robust scaling estimator Q_n (Rousseeuw and Croux, 1993) were applied to define the catchment source tracer property distributions, where Q_n is defined as:

$$Q_n = d\left\{\left|x_i - x_j\right|; i < j\right\}_{(k)}$$

Where d is a constant factor (1.0483), $x_i - x_j$ is the pairwise distances and k = $\left(\frac{n}{2}\right)$ $\approx\left(\frac{n}{2}\right)/4$ where $h=\left(\frac{n}{2}\right)+1$ is roughly half the number of the observations. On the basis of corresponding results for the channel bed sediment sample sets collected from each study catchment, the same procedure was used to define the tracer distributions for measured sediment mixtures.

Table 6.2: The results of the Lilliefors test for Normality using the source and sediment tracer data measured for the Afon Cyneiniog study catchment.

Property	P-value	Property	P-value	Property	P-value
Al	0.001*	Sc	0.001*	Nd	0.001*
Ba	0.114	Co	0.001*	Sm	0.016*
Cr	0.025*	Ga	0.001*	Eu	0.004*
Cu	0.001*	Ge	0.001*	Gd	0.019*
Fe	0.001*	As	0.001*	Tb	0.001*
K	0.314	Rb	0.500	Dy	0.001*
Li	0.013*	Y	0.001*	Ho	0.001*
Mg	0.231	Zr	0.001*	Er	0.001*
Mn	0.001*	Mo	0.001*	Tm	0.001*
Na	0.028*	Cd	0.001*	Yb	0.001*
Ni	0.001*	Sn	0.001*	Lu	0.001*
Pb	0.001*	Sb	0.001*	Hf	0.001*
Sr	0.500	Cs	0.001*	Tl	0.001*
Ti	0.001*	La	0.005*	Bi	0.001*
V	0.015*	Ce	0.001*	U	0.002*
Zn	0.001*	Pr	0.001*		

* statistically significant values at $p \leq 0.05$

Table 6.3: The results of the Lilliefors test for Normality using the source and sediment tracer data measured for the Afon Melindwr study catchment.

Property	P-value	Property	P-value	Property	P-value
Al	0.500	Sc	0.001*	Nd	0.001*
Ba	0.001*	Co	0.001*	Sm	0.001*
Cr	0.003*	Ga	0.021*	Eu	0.001*
Cu	0.001*	Ge	0.001*	Gd	0.001*
Fe	0.500	As	0.003*	Tb	0.001*
K	0.302	Rb	0.020*	Dy	0.003*
Li	0.319	Y	0.122	Ho	0.011*
Mg	0.001*	Zr	0.001*	Er	0.020*
Mn	0.067	Mo	0.001*	Tm	0.026*
Na	0.001*	Cd	0.001*	Yb	0.044*
Ni	0.266	Sn	0.001*	Lu	0.011*
Pb	0.001*	Sb	0.001*	Hf	0.001*
Sr	0.001*	Cs	0.018*	Tl	0.001*

Ti	0.001*	La	0.001*	Bi	0.001*
V	0.001*	Ce	0.001*	U	0.022*
Zn	0.001*	Pr	0.001*		

* statistically significant values at $p \leq 0.05$

A fingerprint property conservation test was applied to the measured tracer data for each individual catchment, based on the mixing space defined by the ranges in the medians of the property values measured for each source type end member (with corrections applied during the mass balance modelling – see section on numerical modelling). This conservation test was used to identify those properties for which the bed sediment sample tracer medians were located in the corresponding end member mixing polygon. Properties outside the mixing polygon violate a fundamental assumption of the numerical modelling and generate unacceptable mixing model performance. An additional preliminary screening of the individual tracer properties for discriminating the fine-grained sediment sources was based on a tracer discriminatory ratio (Table 6.4). This was based on the ratio of between-source to within-source variation. A fundamental requirement of source fingerprinting is that individual properties should have higher between-source than within-source variation (cf. Collins and Walling, 2002; Pulley et al., 2015). In combination, the range test for tracer conservation and the variability ratio screening identified the lists of individual tracers (Table 6.5) that were taken forward into statistical testing.

Table 6.4: Summary information on the variability ratio screening of tracer properties.

	Catchment	Sources	Summary statistics on variance ratio			Threshold	No. of tracer properties
			Q1	median	Q3	ratio used	selected
A	St Lawrence Stream	5	13.1	18.3	22.9	10	34
B	Bolingey Stream	4	10.5	15.7	20.5	10	28
C	Porthleven Stream	5	4.2	6.7	8	5	26
D	Hayle	5	7.3	10	14.7	10	23
E	River Burn(Tavy)	5	9.5	16.1	30.1	10	26
F	Mardle	4	14.4	19.6	22.1	10	31
G	Afon Melindwr	5	8.1	10.7	12.6	10	25
H	Nant Magwr	5	7.8	10	11.6	10	19
I	Afon Cyneiniog	3	4.7	7	13.6	10	14
J	Afon Ystwyth	4	10.3	12.3	15.4	10	14
K	Wye	3	6.1	16.1	28.3	10	22
L	Rea Brook	5	7.5	12.3	16.9	10	29
M	River South Tyne	5	6.7	9.2	12.3	10	19
N	Red Tarn Beck	4	5.3	8.6	13.7	10	14
O	River Greta	4	7.2	16.2	23.7	10	26
P	Arkle Beck	3	4.6	10.9	14.8	10	21
Q	Egglestone Beck	4	7.2	9.8	11.7	5	22
R	Hudeshope Beck	5	5.5	8.3	14.3	10	16
S	Bedburn Beck	4	8.3	14.2	19.2	10	21
T	River East Allen	5	7.2	9	13.8	5	20

Table 6.5: Lists of tracer properties passing the range test and variability ratio thresholds and thereby included in further statistical analyses for the selection of composite signatures for sediment source discrimination.

	Catchment	Properties
A	St Lawrence	As,Bi,Co,Cr,Cu,Dy,Er,Eu,Fe,Gd,Ge,Ho,La,Li,Lu,Mg,Mn,Mo,Nd,Pr,Rb,Sb,Sc,Sm,Sr,Tb,Ti,Tl,Tm,Yb
B	Bolingey Stream	Al,As,Bi,Cr,Dy,Er,Gd,Ge,Ho,La,Li,Mg,Na,Nd,Pb,Pr,Sb,Sc,Sm,Sr,Tb,Ti,Tl,Tm,Yb
C	Porthleven	Ce,Cr,Dy,Er,Eu,Gd,Ge,Ho,La,Li,Lu,Mg,Na,Nd,Ni,Pr,Sm,Sn,Tb,Tl,Tm,Yb,Zr
D	Hayle	As,Ba,Bi,Cr,Dy,Er,Fe,Ga,Ho,Li,Lu,Mg,Na,Pb,Sb,Sr,Tb,Ti,Tm,Yb,Zr
E	River Burn(Tavy)	Ce,Cs,Cu,Dy,Er,Eu,Fe,Gd,Ge,Hf,La,Lu,Mo,Nd,Pr,Sb,Sc,Sm,Sn,Sr,Tl,Tm,Yb
F	Mardle	Al,As,Ba,Bi,Cd,Ce,Co,Cs,Cu,Dy,Er,Eu,Fe,Ga,Lu,Mg,Mo,Na,Pb,Rb,Sb,Sc,Sn,Sr,Ti,Tl,Yb,Zr
G	Afon Melindwr	Ba,Ce,Cr,Dy,Er,Eu,Gd,Ge,La,Lu,Mo,Nd,Ni,Pb,Pr,Sb,Sm,Sn,Sr,Tb,Ti,Yb
H	Nant Magwr	As,Co,Dy,Eu,Ga,Gd,Ge,La,Mo,Pr,Sb,Sc,Sm,Sn,Sr,Tb,Tl,Tm
I	Afon Cyneiniog	Al,Ce,Ge,La,Li,Mg,Na,Nd,Pb,Pr,Sb,Sc,Sm
J	Afon Ystwyth	As,Cs,Cu,Ga,Hf,Pb,Sb,Sc,Sm,Sn,Tl,Zr
K	Wye	Al,Bi,Cd,Cs,Dy,Er,Eu,Gd,Ho,Li,Lu,Mn,Rb,Sb,Sc,Sr,Tb,Tm,Yb,Zn
L	Rea Brook	Al,Ba,Ce,Cr,Cu,Dy,Er,Eu,Ga,Gd,Ge,Ho,La,Lu,Mg,Mo,Nd,Pb,Pr,Sm,Sn,Sr,Tb,Ti,Tm,Yb,Zn
M	River South Tyne	As,Cd,Co,Cs,Ga,Mg,Mn,Na,Ni,Pb,Rb,Sc,Sr,Ti,Zn,Zr
N	Red Tarn Beck	As,Bi,Ga,Gd,Hf,Mo,Nd,Sm,Sn,Sr,Tb,Tm,Zr
O	River Greta	As,Ba,Bi,Ce,Cs,Cu,Dy,Eu,Fe,Ga,Gd,Ge,La,Li,Mg,Mo,Nd,Pb,Pr,Sb,Sm,Sn,Sr,Ti
P	Arkle Beck	As,Bi,Cs,Cu,Er,Eu,Ho,Li,Mo,Ni,Rb,Sb,Sc,Sn,Tm,Yb,Zn,Zr
Q	Egglestone Beck	Al,As,Cs,Cu,Dy,Eu,Fe,Ga,Gd,Li,Mg,Na,Pb,Rb,Sb,Sc,Sr,Tm,Zr
R	Hudeshope Beck	Al,As,Cs,Cu,Ga,Li,Mg,Na,Ni,Rb,Sc,Sr,Ti,Tm
S	Bedburn Beck	Al,As,Cs,Cu,Dy,Er,Eu,Ga,Gd,Li,Na,Pb,Rb,Sb,Sc,Sr,Ti,Zn,Zr
T	River East Allen	As,Bi,Cs,Cu,Dy,Er,Gd,Hf,Ho,Mg,Na,Ni,Sr,Tb,Ti,Tm,Yb,Zr

The statistical verification of the discrimination of the sediment source type end members involved the use of the Kruskal-Wallis H-test (KW-H), Principal Component Analysis (PCA) and genetic algorithm-driven Discriminant Function Analysis (GA-DFA) as outlined in Collins et al. (2012c). For the KW-H test, the Chi-square and p-value for each geochemical tracer passing the mass conservation and tracer variability screening (Table 6.5) were used to select properties (see examples in Tables 6.6 and 6.7 for the Afon Cyneiniog and Afon Melindwr study catchments, respectively). The corresponding KW-H outputs for the remainder of the study catchments are presented in Appendix A (Tables A19-A36). The use of PCA for selecting geochemical tracers with the highest ranked loadings provided a basis for verifying additional optimum composite signatures (see example PCA outputs for the Afon Cyneiniog and Afon Melindwr study catchments in Tables 6.8 and 6.9, respectively). In these examples, two components were sufficient for explaining up to 99.9% of the variance. The corresponding PCA outputs for the remainder of the study catchments are presented in Appendix A (Tables A37-A54). In these cases, two components were sufficient for explaining up to 100% of the variance.

Table 6.6: KW-H results for the Afon Cyneiniog study catchment.

Property	H-value	p-value	Property	H-value	p-value
Pb	19.8	0.000	Sb	21.1	0.000
Na	11.7	0.003	K	9.4	0.009
Li	17.3	0.000	La	17.1	0.000
Al	17.2	0.000	Ce	15.8	0.000
Pr	17.1	0.000	Ge	14.4	0.001
Nd	15.0	0.001	Sm	8.2	0.016
Mg	11.4	0.003	Sc	13.2	0.001

Table 6.7: KW-H results for the Afon Melindwr study catchment.

Property	H-value	p-value	Property	H-value	p-value
Ba	21.9	0.000	Ni	22.3	0.000
Ce	14.1	0.007	Pb	16.9	0.002
Cr	25.0	0.000	Pr	19.3	0.001
Dy	22.0	0.000	Sb	15.6	0.004
Er	21.1	0.000	Sm	14.6	0.006
Eu	17.2	0.002	Sn	28.6	0.000
Gd	12.5	0.014	Sr	26.7	0.000
Ge	25.5	0.000	Tb	29.9	0.000
K	14.8	0.005	Ti	31.2	0.000
La	19.2	0.001	U	29.2	0.000
Lu	26.7	0.000	Y	24.2	0.000
Mo	29.4	0.000	Yb	22.3	0.000
Nd	18.1	0.001			

Table 6.8: Highest ranked property loadings provided by the outputs of the PCA for the Afon Cyneiniog study catchment.

Property	PC-1[a]	Property	PC-2[b]
Pb	0.937	Al	0.893
Al	0.348	Pb	0.340
K	0.034	Mg	0.290
Mg	0.022	K	0.052
Na	0.005	Ce	0.006
Sb	0.004	Na	0.005
Ce	0.003	Li	0.004
Nd	0.002	Nd	0.003
La	0.002	La	0.003
Li	0.001	Pr	0.001
Pr	0.000	Ge	0.001
Ge	0.000	Sm	0.000
Sm	0.000	Sc	0.000
Sc	0.000	Sb	0.000
VE%	88.2	VE%	10.6

[a] Principal Component 1; [b] Principal Component 2; VE % variance explained

Table 6.9: Highest ranked property loadings provided by the outputs of the PCA for the Afon Melindwr study catchment.

Property	PC-1[a]	Property	PC-2[b]
Pb	1.000	K	0.993
Sb	0.006	Ti	0.116
K	0.006	Ce	0.013
Ti	0.005	La	0.008
Ba	0.001	Nd	0.008
Ce	0.001	Sb	0.006
Ni	0.001	Sr	0.006
Nd	0.000	Pb	0.005
La	0.000	Ni	0.003
Sr	0.000	Cr	0.003
Pr	0.000	Pr	0.002
Cr	0.000	Ge	0.002
Ge	0.000	Sn	0.002
Sm	0.000	Ba	0.001
Gd	0.000	Sm	0.001
Mo	0.000	Gd	0.001
Y	0.000	Y	0.001
Sn	0.000	Mo	0.001
U	0.000	U	0.000
Eu	0.000	Dy	0.000
Yb	0.000	Eu	0.000
Tb	0.000	Tb	0.000
Er	0.000	Yb	0.000
Dy	0.000	Er	0.000
Lu	0.000	Lu	0.000
VE%	99.5	VE%	0.4

[a] Principal Component 1; [b] Principal Component 2; VE % variance explained

Final composite signatures were identified using the highest ranked properties from either the KW-H test or PCA outputs for each study catchment. Each individual tracer for each composite fingerprint as well as the property sets in their entirety, were passed through the DFA to calculate the tracer discriminatory weightings and the total discriminatory efficiency of each set of properties. Examples of final signatures based on the KW-H test are provided in Tables 6.10 and 6.11 for the Afon Cyneiniog and Afon Melindwr study catchments, respectively. The corresponding results for the additional study catchments are presented in Tables A55-A72 in Appendix A. The KW-H optimum signatures presented in Tables 6.10 and 6.11, as examples, correctly distinguished between 73% and 90% of the source type end member samples. In the case of the remaining study catchments, the KW-H optimum fingerprints classified between 74-100% of the source type samples into the correct end members (Tables A55-A72 in Appendix A). Examples of the final composite signatures selected using PCA are also provided in Tables 6.10 and 6.11. For these two examples, the PCA optimum geochemical signatures correctly classified between 88% (Afon Melindwr) and 91% (Afon Cyneiniog) of the source samples. For the additional study catchments, the optimum geochemical signatures selected using PCA correctly distinguished between 68-93% of the catchment source type end member samples (Tables A55-A72 in Appendix A).

Table 6.10: The final composite signatures selected using KW-H and PCA for the Afon Cyneiniog study catchment.

KW-H			PCA		
Property	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**
Al	61	1.11	Al	61	1.43
La	67	1.22	Ce	64	1.50
Li	67	1.22	K	42	1.00
Pb	61	1.11	Mg	64	1.50
Pr	64	1.17	Na	64	1.50
Sb	55	1.00	Pb	61	1.43
Total[3]	73		Total[3]	91	

[1] % source type end member samples classified correctly by individual properties
[2] tracer discriminatory weighting used in the mass balance modelling
[3] % source type end member samples classified correctly by composite signature

Table 6.11: The final composite signatures selected using KW-H and PCA for the Afon Melindwr study catchment.

KW-H			PCA		
Property	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**
Lu	45	1.16	Ba	42	1.12
Mo	43	1.12	Ce	37	1.00
Sn	46	1.18	K	42	1.13
Sr	52	1.33	La	41	1.10
Tb	53	1.36	Pb	54	1.44
Ti	49	1.28	Sb	42	1.14
U	39	1.00	Ti	49	1.32
Total[3]	90		Total[3]	88	

[1] % source type end member samples classified correctly by individual properties
[2] tracer discriminatory weighting used in the mass balance modelling
[3] % source type end member samples classified correctly by composite signature

As examples, Tables 6.12 and 6.13 present the results of the GA-DFA for the Afon Cyneiniog and Afon Melindwr catchments, respectively. The GA-DFA procedure was used to identify three optimum composite signatures for each study catchment (see results tables for remaining study catchments in Appendix A – Tables A73-A90). Each GA-DFA optimum signature was selected on the basis of 200 repeat iterations, using the minimisation of Wilks' lambda as a stepwise selection algorithm and a probability value for parameter entry of 0.05. GA-DFA was used as a basis for assessing the percentage of the source type end member samples discriminated correctly by each individual geochemical tracer selected for each optimum composite signature, as well as the total discriminatory efficiency of the property sets (Tables 6.12 and 6.13 as examples). In the case of the examples in Tables 6.12 and 6.13, the total discriminatory power of the GA-DFA composite signatures ranged from 91% (GA-DFA run 2) to 94% (GA-DFA runs 1,3) for the Afon Cyneiniog and from 96% (GA-DFA run 2) to 98% (GA-DFA runs 1, 3) for the Afon Melindwr catchment. The relative discriminatory efficiency of the individual geochemical tracers comprising each composite signature was used to provide tracer discriminatory weightings for the mass balance modelling (see examples in Tables 6.12 and 6.13). These values were calculated using the ratio of the proportion of the source type end member samples classified correctly by any

individual tracer to the corresponding value for the property in the same signature with the lowest discriminatory efficiency. The latter were therefore assigned tracer discriminatory weightings of 1.0. Between 90-100% of the source type end member samples collected in the remaining 18 study catchments were classified correctly on the basis of the final signatures selected using GA-DFA (Tables A73-A90 in Appendix A).

Table 6.12: The results of GA-DFA for the Afon Cyneiniog study catchment.

GA-DFA 1			GA-DFA 2			GA-DFA 3		
Property	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**
Al	61	1.43	K	42	1.00	K	42	1.00
K	42	1.00	Li	67	1.57	Li	67	1.57
Pb	61	1.43	Sb	55	1.29	Sb	55	1.29
Pr	64	1.50	Sc	67	1.57	Sc	67	1.57
Sc	67	1.57	Sm	48	1.14	Sm	48	1.14
Total[3]	94		Total[3]	91		Total[3]	91	

[1] % source type end member samples classified correctly by individual properties
[2] tracer discriminatory weighting used in the mass balance modelling
[3] % source type end member samples classified correctly by composite signature

Table 6.13: The results of GA-DFA for the Afon Melindwr study catchment.

GA-DFA 1			GA-DFA 2			GA-DFA 3		
Property	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**
Ba	42	1.00	Ba	42	1.00	Cr	52	1.52
Dy	44	1.06	Dy	44	1.06	Dy	44	1.30
Mo	43	1.04	Mo	43	1.04	Gd	36	1.07
Nd	42	1.01	Pb	54	1.29	Mo	43	1.27
Pb	54	1.29	Sr	52	1.23	Pb	54	1.58
Sb	42	1.02	Tb	53	1.26	Sb	42	1.25
Tb	53	1.26				Sm	34	1.00
						Tb	53	1.55
Total[3]	98		Total[3]	96		Total[3]	98	

[1] % source type end member samples classified correctly by individual properties
[2] tracer discriminatory weighting used in the mass balance modelling
[3] % source type end member samples classified correctly by composite signature

6.1.4 Mass balance modelling for sediment source apportionment and uncertainty analysis

The numerical mass balance model described in Collins et al. (2010a) was used to apportion the relative contributions from the source type end members in each of the 20 catchments. This model is based on the following algorithm:

$$\sum_{i=1}^{n}\left\{\left(C_i-\left(\sum_{s=1}^{m}P_sS_{si}Z_sSV_{si}\right)\right)/C_i\right\}^2 W_i \quad (1)$$

where:

C_i = deviate median concentration of fingerprint property (i) in channel bed sediment sample;

P_s = the optimised percentage contribution from source type end member (s);

S_{si} = deviate median concentration of fingerprint property (i) in source type end member (s);

Z = particle size correction factor for source type end member (s);

SV_{si} = weighting representing the within-source variation of geochemical tracer (i) in source type end member (s);

W_i = tracer discriminatory weighting;

n = number of geochemical tracers comprising the optimum composite fingerprint;

m = number of source type end members.

Detailed descriptions of the various components of the mass balance model are provided in Collins et al. (1997, 2010a, 2012c). The work reported here used some of the latest revisions to this model; the within-source variation and tracer discriminatory efficiency weightings. The former weighting is included in the algorithm to ensure that those geochemical tracers with smaller variance exert more influence on the solutions generated for any specific composite signature. This weighting is based on the inverse of the coefficient of variation for individual tracers. The basis for estimating the tracer discriminatory power weighting is provided in the previous section detailing statistical analyses. For the work reported here, no upper boundary constraint of 0.5 (as opposed to 1.0 used for all other end members) was used as prior information on the potential relative contribution of channel banks (cf. Collins et al., 2010a) to in-stream sediment mixtures since this prior was based on a strategic dataset not including headwater metal mine catchments. The mass balance model uses median tracer values in the source end members and corresponding instream sediment as input and uncertainties in characterising these values on the basis of relatively few samples were quantified explicitly using the scaling of the tracer parameter distributions (probability density functions; pdfs) based on Q_n and a Monte Carlo framework. The Monte Carlo framework included local optimisation of the model repeat solutions. Repeat solutions using the local optimisation (30,000 for each composite fingerprint identified for each study catchment) were generated using a stratified approach (Latin Hypercube) to sampling the input tracer distributions.

Uncertainty associated with the predicted sediment source proportions was assessed in the following ways, viz:

- quantifying the full ranges in the predicted deviate median source proportions as represented by the output pdfs generated by the mass balance model and using these to estimate relative frequency-weighted average median inputs (*R*) from the individual sediment sources.

$$R = \sum_{i=1}^{n} v_i \; F_i \qquad (2)$$

  where *n* is the number of intervals for the predicted deviate relative contribution, scaled between 0 and 1; and *v* and *F* are the mid-value and the relative frequency for the *ith* interval, respectively (Collins et al., 2012c).

- quantifying the convergence of the model solutions and their precision by calculating 95% confidence limits about the average median source proportions, using 10 sets of 30,000 repeat iterations for one composite signature identified for each study catchment
- assessing the goodness-of-fit (GOF) as represented by the relative mean error squared (RMES) between the source-weighted predicted sediment chemistry and the measured signatures of the sampled channel bed sediment, using:

$$GOF_{RMES} = 1 - \left[\frac{1}{n} * \sum_{i=1}^{n} \left\{ \left(C_i - \left(\sum_{s=1}^{m} P_s S_{si} Z_s SV_{si} \right) \right) / C_i \right\}^2 W_i \right] \qquad (3)$$

  where *n* is the number of tracer properties comprising any composite signature. This GOF estimator has been widely used in sediment source fingerprinting studies (Motha et al., 2003; Collins et al., 2010a; Haddachi et al., 2013).
- assessing the goodness-of-fit (GOF) as represented by the absolute relative mean error (ARME) between the source-weighted predicted sediment chemistry and the measured signatures of the sampled channel bed sediment, using:

$$GOF_{ARME} = 1 - SQRT \left[\sum_{i=1}^{n} \left\{ \left(C_i - \left(\sum_{s=1}^{m} P_s S_{si} Z_s SV_{si} \right) \right) / C_i \right\}^2 W_i \right] / n \qquad (4)$$

  where *n* is the number of tracer properties comprising any composite signature. This GOF estimator has also been used previously (e.g. Collins et al., 1997).

6.2 Results

Previous sediment source fingerprinting studies have generally used a threshold of 15-20% error in the assessment of the goodness-of-fit (GOF) between predicted source-weighted and measured sediment geochemistry (*cf.* Walling and Collins, 2000). Table 6.14 shows that nearly all statistically-verified composite signatures identified for the 20 catchments generated acceptable (GOF ≥0.8) goodness-of-fit estimates on the basis of equation 3. The exceptions were the composite signatures identified on the basis of PCA for the Afon Cyneiniog catchment, KW-H, PCA and GA-DFA2 for Porthleven Stream and GA-DFA2 for Rea Brook (Table 6.14). As an additional evaluation of the modelled estimates of source proportions, equation 4 was also used. Here, the results in Table 6.14 confirmed that more

of the mixing model source-weighted predictions of channel bed sediment geochemistry failed to return an acceptable (≥0.8) GOF. Most noticeably, the numerical mass balance modelling using the five composite signatures for the Rea Brook study catchment failed to return source-weighted predictions of sediment geochemistry with an acceptable GOF to values measured in the laboratory (Table 6.14).

Table 6.15 presents the relative frequency-weighted average median source proportions predicted by the mass balance modelling using each of the five composite signatures identified for each of the 20 catchments. The final weighted-average median source proportions (Table 6.15) were generated taking the modelled predictions for signatures returning acceptable (≥0.8) GOF estimates using both the RMSE and ARME estimators and weighting these on the basis of the absolute relative error and % discriminatory power associated with each composite signature. These weighted-average estimates should be taken as the final predictions of sediment source proportions. Here, it is important to note that composite signatures yielding acceptable estimates of GOF can generate contrasting source apportionment for any given catchment (Table 6.15). This reflects the equifinality associated with source fingerprinting procedures and underscores the need to base final source estimates on multiple, rather than single, composite signatures (Collins et al., 2012c, 2013, 2014). The final source apportionment estimates are, however, weighted to reflect the GOF and discriminatory power afforded by the individual composite signatures deemed acceptable for any given location. Further analysis using artificial mixtures would be needed to test the final source apportionment estimates. Given the poor performance of the source-weighted predictions of sediment geochemistry for Rea Brook, on the basis of ARME, no reliable source proportions could be generated for this study catchment.

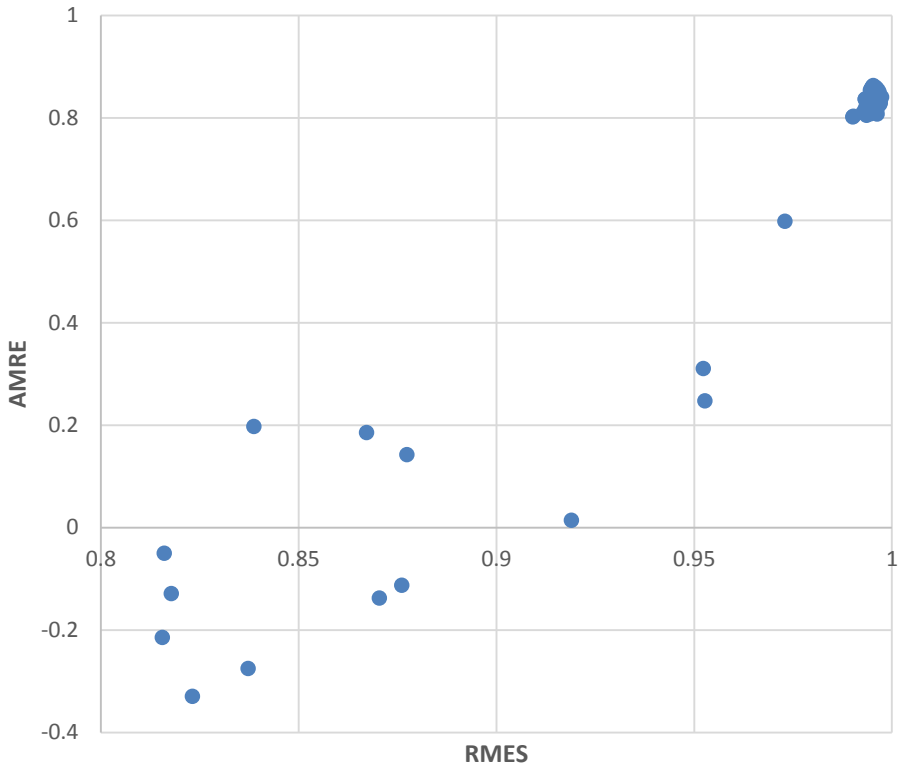


Figure 6.1: Plot of AMRE versus RMES for all modelled artificial channel bed sediment mixtures.

Table 6.14: The relative mean error squared (RMES) and absolute relative mean error (ARME) associated with the predicted source proportions using each composite signature identified for each study catchment.

Catchment	Signature	RMES	ARME	Catchment	Signature	RMES	ARME
A St Lawrence Stream	KW-H*	0.82	-0.13	K Wye	KW-H	0.99	0.80
	PCA	1.00	0.82		PCA	0.99	0.84
	GA - 1	1.00	0.83		GA - 1	0.99	0.80
	GA - 2	1.00	0.85		GA - 2	0.99	0.84
	GA - 3	1.00	0.85		GA - 3*	0.84	0.20
B Bolingey Stream	KW-H	1.00	0.85	L Rea Brook	KW-H*	0.95	0.31
	PCA	1.00	0.86		PCA*	0.88	-0.11
	GA – 1	1.00	0.85		GA - 1*	0.82	-0.21
	GA – 2	0.99	0.83		GA - 2*	0.79	-0.44
	GA – 3	1.00	0.84		GA - 3*	0.84	-0.28
C Porthleven Stream	KW-H*	0.70	-0.45	M River South Tyne	KW-H	1.00	0.82
	PCA*	0.71	-0.43		PCA	1.00	0.83
	GA - 1	1.00	0.84		GA - 1	1.00	0.83
	GA - 2*	0.74	-0.45		GA - 2	1.00	0.84
	GA - 3	1.00	0.82		GA - 3*	0.82	-0.33
D Hayle	KW-H	1.00	0.84	N Red Tarn Beck	KW-H	0.99	0.82
	PCA	1.00	0.82		PCA	1.00	0.84
	GA - 1	1.00	0.83		GA - 1	0.99	0.83
	GA - 2	1.00	0.83		GA - 2*	0.97	0.60
	GA - 3	1.00	0.82		GA - 3	0.99	0.80
E River Burn (Tavy)	KW-H	1.00	0.84	O River Greta	KW-H	1.00	0.84
	PCA	0.99	0.81		PCA	1.00	0.83
	GA - 1	1.00	0.84		GA - 1	1.00	0.83
	GA - 2	1.00	0.85		GA - 2	1.00	0.86
	GA - 3	1.00	0.83		GA - 3	1.00	0.83
F Mardle	KW-H*	0.95	0.25	P Arkle Beck	KW-H	0.99	0.86
	PCA*	0.92	0.01		PCA	0.99	0.85
	GA - 1	1.00	0.84		GA - 1	1.00	0.86
	GA - 2*	0.80	-0.56		GA - 2	1.00	0.86
	GA - 3*	0.81	-0.53		GA - 3	1.00	0.86
G Afon Melindwr	KW-H	1.00	0.83	Q Egglestone Beck	KW-H	1.00	0.83
	PCA	0.99	0.81		PCA	1.00	0.84
	GA – 1	1.00	0.84		GA – 1	1.00	0.84
	GA – 2	1.00	0.85		GA - 2	1.00	0.83
	GA – 3	1.00	0.83		GA - 3	1.00	0.83
H Nant Magwr	KW-H	1.00	0.83	R Hudeshope Beck	KW-H	1.00	0.84
	PCA	1.00	0.83		PCA	1.00	0.84
	GA - 1	0.99	0.82		GA - 1	1.00	0.83
	GA - 2	1.00	0.83		GA - 2	1.00	0.84
	GA - 3	1.00	0.84		GA - 3	0.99	0.82
I Afon Cyneiniog	KW-H	1.00	0.84	S Bedburn Beck	KW-H	1.00	0.84
	PCA*	-1.60	-2.95		PCA*	0.87	0.19
	GA – 1	1.00	0.85		GA – 1	1.00	0.84
	GA – 2	0.99	0.81		GA – 2	1.00	0.85
	GA – 3	1.00	0.85		GA – 3	1.00	0.85
J Afon Ystwyth	KW-H	0.99	0.82	T River East Allen	KW-H	1.00	0.81
	PCA*	0.88	0.14		PCA*	0.87	-0.14
	GA - 1*	0.82	-0.05		GA - 1	1.00	0.83
	GA - 2	0.99	0.82		GA - 2	1.00	0.84
	GA - 3	0.99	0.82		GA - 3	1.00	0.83

* model runs returned acceptably low ARME

In the case of the mine waste sources, the final estimates of predicted relative contributions to the fine-grained channel bed sediment ranged from 3% in the River Greta to 26% in the Red Tarn Beck catchment. Within this overall range, reasonably high relative contributions were also predicted for the River East Allen (19%), Hayle (18%), Egglestone Beck (16%) and Bolingey Stream (16%) study catchments (Table 6.15). Fine-grained sediment inputs from the dis-used mine workings at the remaining sites were lower, reflecting a combination of the coarse grain-size of the mine workings/waste, the development of protective vegetation cover and the insertion of pollution mitigation measures including settling ponds.

The contributions from urban street dust ranged from 3% for the Hudeshope Beck catchment to 61% for Red Tarn Beck (Table 6.15). This source category was also predicted to be important for the Hayle (35%) and Nant Magwr (22%) catchments (Table 6.15). Urban street dust along roadsides in rural settlements and besides road drains has a strong chance of being delivered into neighbouring streams due to the connectivity provided by rural road drain networks.

In the case of eroding channel banks and subsurface sources, the corresponding contributions across the 20 catchments ranged from 5% in Red Tarn Beck to 76% for Porthleven Stream. High contributions were also estimated for the Mardle (67%), Arkle Beck (62%), St Lawrence Stream (54%) and Hudeshope Beck (52%) catchments. Given the juxtaposition of eroding channel banks to streams, mobilised material is readily delivered into the watercourse in contrast to some of the fine-grained sediment mobilised from more distal catchment sediment sources (cf. Collins and Walling, 2004). For the majority of the 20 catchments, the relative contribution of fine-grained sediment from eroding channel banks/subsurface sources was less than 50% and therefore in agreement with the national data for river catchments previously reported by Walling and Collins (2005).

Recent work using sediment source tracing procedures has identified that damaged road verges can make important contributions to fine-grained sediment pressures in river catchments across the UK (Collins et al., 2010b, 2012b). These areas of river catchments are increasingly damaged by the growing volume of road traffic in rural areas, larger farm machinery, as well as by livestock moving between fields or between fields and milking parlours on a regular basis. Due to the low surface roughness of metalled roads, the trampled material is readily mobilised by rainstorms, with as little as 5 mm of rain mobilising material, which is readily delivered to streams via road drains and river crossings. In the case of the 20 catchments used for source apportionment work, the final estimates of the relative contributions from damaged road verges ranged from 8% for Porthleven Stream to 44% for the Afon Melindwr catchment. The fact that the highest estimates of sediment contributions from damaged road verges were not predicted for the two catchments with the highest rural road density (St Lawrence Stream – 2 km/km^2; Bolingey Stream – 1.3 km/km^2) underscores that additional factors are at play in governing sediment loss from this source end member. The damaged road verge contribution in the latter study area (25%) was, however, reasonably high within the range across the study catchments (Table 6.15). The lower contribution from damaged road verges predicted for the Porthleven Stream is, in part, likely to reflect the low number (6) of river crossings identified in this catchment. More river crossing (10) were identified in the Afon Melindwr catchment and this is likely to have contributed to the higher predicted contribution from damaged road verges (Table 6.15). Although the highest number of river crossings (41) was recorded in the Arkle Beck catchment, damaged road verges were not identified as a sediment source in this locality.

Nevertheless, the high number of river crossings in Arkle Beck is likely to be playing an important role in the delivery of mobilised fine-grained sediment to the channel network.

Fine-grained sediment loss from eroding agricultural topsoils was estimated to contribute between 3% (River Mardle) and 85% (Afon Cyneiniog) of the fine-grained channel bed sediment sampled at the catchment outlets. In the case of the River Mardle, ~53% of the land cover is rough grazing, where lower stocking densities can sometimes be expected to avoid widespread severe soil erosion and sediment mobilisation. This is borne out by the fact that the catchment with the largest proportion of rough grazing (~93% - Red Tarn Beck) was also predicted to have a low sediment contribution (8% - Table 6.15) from eroding agricultural topsoils. A number of studies have reported problems of soil erosion and sediment loss to rivers in association with higher stocking densities on improved grassland and consequential widespread soil pugging, poaching and compaction (Trimble and Mendel, 1995; Evans, 1998; Singleton et al., 2000; Kurz et al., 2006; Drewry et al., 2008; Collins et al., 2010b, 2012b). The important agricultural topsoil contributions in the Afon Cyneiniog (86%), Wye (71%) and Nant Magwr (41%) catchments (Table 6.15) are likely to reflect the importance of improved pasture for local land cover (~59%, ~78%, ~68%). Again, however, the much lower predicted contribution from the agricultural topsoil end member estimated for the St Lawrence Stream (11%) belies the importance of improved grazing for local land cover (~59%), revealing the complexity of controls on catchment sediment dynamics. Although soil erosion and sediment loss are important issues associated with arable land cover and cropping practices, the two catchments with the highest proportion of arable land (Porthleven Stream – 25% and the River Hayle – 27%) were not associated with high relative contributions from the agricultural source end member (9% and 13%, respectively – Table 6.15).

Table 6.15: Estimated relative frequency-weighted median sediment source contributions in each study catchment.

* Estimated using a weighting combining the absolute relative error and % discriminatory power associated with each composite signature

Catchment	Signature	Agricultural topsoils	Damaged road verges	Channel banks/ subsurface sources	Urban street dust	Mine Waste
A St Lawrence Stream	KW-H	0.03	0.22	0.64	0.03	0.08
	PCA	0.03	0.48	0.43	0.03	0.03
	GA – 1	0.03	0.13	0.68	0.03	0.13
	GA – 2	0.33	0.04	0.30	0.05	0.28
	GA – 3	0.03	0.03	0.74	0.10	0.10
	Weighted average*	***0.11***	***0.15***	***0.54***	***0.06***	***0.14***
B Bolingey Stream	KW-H	0.07	0.20	0.57	-	0.16
	PCA	0.27	0.37	0.23	-	0.13
	GA – 1	0.31	0.19	0.27	-	0.23
	GA – 2	0.06	0.40	0.35	-	0.19
	GA – 3	0.53	0.12	0.26	-	0.09
	Weighted average*	***0.25***	***0.25***	***0.34***	***-***	***0.16***
C Porthleven Stream	KW-H	0.03	0.03	0.88	0.03	0.03
	PCA	0.88	0.03	0.03	0.03	0.03
	GA – 1	0.05	0.07	0.82	0.03	0.03
	GA – 2	0.03	0.38	0.53	0.03	0.03
	GA – 3	0.14	0.10	0.69	0.03	0.04
	Weighted average*	***0.09***	***0.08***	***0.76***	***0.03***	***0.04***
D Hayle	KW-H	0.03	0.03	0.63	0.28	0.03
	PCA	0.25	0.03	0.03	0.41	0.28
	GA – 1	0.11	0.05	0.20	0.46	0.18
	GA – 2	0.11	0.18	0.17	0.34	0.20
	GA – 3	0.13	0.20	0.17	0.27	0.23
	Weighted average*	***0.13***	***0.10***	***0.24***	***0.35***	***0.18***
E River Burn (Tavy)	KW-H	0.12	0.41	0.08	0.29	0.10
	PCA	0.03	0.66	0.03	0.03	0.25
	GA – 1	0.43	0.24	0.19	0.04	0.10
	GA – 2	0.34	0.32	0.18	0.05	0.11
	GA – 3	0.28	0.35	0.27	0.05	0.05
	Weighted average*	***0.25***	***0.38***	***0.16***	***0.09***	***0.12***
F Mardle	KW-H	0.32	0.13	0.52	-	0.03
	PCA	0.03	0.47	0.47	-	0.03
	GA – 1	0.03	0.22	0.67	-	0.08
	GA – 2	0.67	0.22	0.03	-	0.08
	GA – 3	0.03	0.56	0.38	-	0.03
	Weighted average*	***0.03***	***0.22***	***0.67***	***-***	***0.08***

Catchment	Signature	Agricultural topsoils	Damaged road verges	Channel banks/ subsurface sources	Urban street dust	Mine Waste
G Afon Melindwr	KW-H	0.25	0.40	0.18	0.04	0.13
	PCA	0.13	0.74	0.04	0.04	0.05
	GA – 1	0.36	0.37	0.07	0.13	0.07
	GA – 2	0.28	0.40	0.11	0.12	0.09
	GA – 3	0.36	0.33	0.04	0.21	0.06
	Weighted average*	***0.28***	***0.44***	***0.09***	***0.11***	***0.08***
H Nant Magwr	KW-H	0.44	0.32	0.13	0.08	0.03
	PCA	0.18	0.27	0.03	0.49	0.03
	GA – 1	0.43	0.11	0.04	0.28	0.14
	GA – 2	0.42	0.36	0.04	0.10	0.08
	GA – 3	0.55	0.21	0.04	0.17	0.03
	Weighted average*	***0.41***	***0.25***	***0.06***	***0.22***	***0.06***
I Afon Cyneiniog	KW-H	0.89	-	0.08	-	0.03
	PCA	0.94	-	0.03	-	0.03
	GA – 1	0.81	-	0.03	-	0.16
	GA – 2	0.92	-	0.05	-	0.03
	GA – 3	0.83	-	0.07	-	0.10
	Weighted average*	***0.86***	-	***0.06***	-	***0.08***
J Afon Ystwyth	KW-H	0.30	0.30	0.35	-	0.05
	PCA	0.03	0.22	0.72	-	0.03
	GA – 1	0.38	0.42	0.17	-	0.03
	GA – 2	0.45	0.26	0.26	-	0.03
	GA – 3	0.21	0.52	0.12	-	0.15
	Weighted average*	***0.32***	***0.36***	***0.24***	***-***	***0.08***
K Wye	KW-H	0.82	-	0.13	-	0.05
	PCA	0.47	-	0.47	-	0.06
	GA – 1	0.94	-	0.03	-	0.03
	GA – 2	0.62	-	0.33	-	0.05
	GA – 3	0.84	-	0.03	-	0.13
	Weighted average*	***0.71***	***-***	***0.24***	***-***	***0.05***
L Rea Brook	KW-H	0.83	0.03	0.03	0.03	0.08
	PCA	0.35	0.03	0.46	0.03	0.13
	GA – 1	0.83	0.03	0.03	0.03	0.08
	GA – 2	0.54	0.03	0.32	0.03	0.08
	GA – 3	0.74	0.03	0.17	0.03	0.03
	Weighted average*	***NR***	***NR***	***NR***	***NR***	***NR***
M River South Tyne	KW-H	0.29	0.42	0.17	0.07	0.05
	PCA	0.56	0.14	0.17	0.10	0.03
	GA – 1	0.47	0.24	0.20	0.05	0.04
	GA – 2	0.33	0.40	0.16	0.07	0.04
	GA – 3	0.63	0.28	0.03	0.03	0.03
	Weighted average*	***0.41***	***0.30***	***0.18***	***0.07***	***0.04***

Catchment	Signature	Agricultural topsoils	Damaged road verges	Channel banks/ subsurface sources	Urban street dust	Non-coal mine
N Red Tarn Beck	KW-H	0.03	-	0.03	0.61	0.33
	PCA	0.28	-	0.11	0.25	0.36
	GA – 1	0.03	-	0.06	0.50	0.41
	GA – 2	0.03	-	0.03	0.86	0.08
	GA – 3	0.03	-	0.03	0.77	0.17
	Weighted average*	***0.08***	***-***	***0.05***	***0.61***	***0.26***
O River Greta	KW-H	0.61	-	0.28	0.08	0.03
	PCA	0.46	-	0.45	0.06	0.03
	GA – 1	0.62	-	0.31	0.04	0.03
	GA – 2	0.65	-	0.23	0.08	0.04
	GA – 3	0.62	-	0.31	0.04	0.03
	Weighted average*	***0.60***	***-***	***0.31***	***0.06***	***0.03***
P Arkle Beck	KW-H	0.16	-	0.77	-	0.07
	PCA	0.30	-	0.50	-	0.20
	GA – 1	0.34	-	0.56	-	0.10
	GA – 2	0.20	-	0.74	-	0.06
	GA – 3	0.33	-	0.52	-	0.15
	Weighted average*	***0.27***	***-***	***0.62***	***-***	***0.11***
Q Egglestone Beck	KW-H	0.27	0.30	0.35	-	0.08
	PCA	0.35	0.18	0.24	-	0.23
	GA – 1	0.45	0.12	0.24	-	0.19
	GA – 2	0.46	0.18	0.25	-	0.11
	GA – 3	0.45	0.07	0.30	-	0.18
	Weighted average*	***0.40***	***0.17***	***0.27***	***-***	***0.16***
R Hudeshope Beck	KW-H	0.45	0.09	0.39	0.04	0.03
	PCA	0.40	0.10	0.41	0.04	0.05
	GA – 1	0.09	0.29	0.56	0.03	0.03
	GA – 2	0.13	0.19	0.60	0.04	0.04
	GA – 3	0.03	0.30	0.59	0.03	0.05
	Weighted average*	***0.20***	***0.21***	***0.52***	***0.03***	***0.04***
S Bedburn Beck	KW-H	0.46	0.22	0.28	-	0.04
	PCA	0.17	0.33	0.47	-	0.03
	GA – 1	0.20	0.20	0.57	-	0.03
	GA – 2	0.24	0.18	0.55	-	0.03
	GA – 3	0.31	0.28	0.32	-	0.09
	Weighted average*	***0.30***	***0.22***	***0.43***	***-***	***0.05***
T River East Allen	KW-H	0.53	0.04	0.31	0.04	0.08
	PCA	0.46	0.03	0.40	0.03	0.08
	GA – 1	0.37	0.09	0.23	0.03	0.28
	GA – 2	0.54	0.08	0.13	0.07	0.18
	GA – 3	0.33	0.31	0.13	0.03	0.20
	Weighted average*	***0.44***	***0.13***	***0.20***	***0.04***	***0.19***

6.2.1 Limitations:

- Because of the reconnaissance nature of the study, driven by the need to cover 20 study rivers, the results are necessarily based on limited numbers of composite samples of both fine-grained interstitial sediment and potential source materials for any individual location. However, the general consistency of the results obtained for the individual rivers within the context of existing understanding of catchment fine-grained sediment sources for the UK (e.g. Walling and Collins, 2005), lends weight to the findings.
- It should be recognised that the provenance of the fine-grained channel bed sediment could vary seasonally, in response to the seasonal pattern of land use practices and the overall hydrological regimes of the study rivers. The representativeness of the sediment provenance data obtained from this study might therefore have been biased by the timing of the river sampling and the fact that a single sediment sampling campaign was undertaken. Some international peer reviewed studies have, however, argued that single visit channel bed sediment sampling at downstream depositional sites in rivers can be used as a reliable means of characterising bed-sediment associated geochemistry (cf. Gilliom et al., 1995; Horowitz et al., 2012).
- River sediment samples for this study were collected using an established remobilisation technique. An alternative approach would be to use time-integrating suspended sediment samplers which are widely used for source tracing studies. These require Flood Defence Consents and so in the context of the number of study areas, these passive samplers were discounted. Time-integrating samplers sample sediment fluxes continuously and so provide temporally representative data.
- The bed sediment source data were not linked to corresponding information on the amounts of fine-grained sediment accumulating in the river channel gravels. It was therefore not possible to comment on potential contrasts in the severity of the siltation/fine-grained sediment problem between the different study rivers and to link such contrasts to the importance of particular catchment sediment sources.
- Equally, it must be recognised that the results presented relate to the relative importance of the potential catchment sediment sources. Thus, it is possible that the actual amount of sediment contributed by a particular source could be greater in one study area than another, even though the relative importance of that source was predicted by the exercise reported here to be lower.
- The sourcing estimates reported here are only based on a local search tool rather than a comparison of both a local and global search tool (cf. Collins et al., 2012b). This was due to the late arrival of the fingerprinting data in the context of the project final reporting deadline and the computational time needed to run global searches using source tracing data. It should be noted, nonetheless, that recent studies have shown that local search tools alone can perform well for source tracing exercises (cf. Collins et al., 2012c).
- For this study, contemporary river channel bed sediment was collected from a single depositional reach at the downstream end of each catchment. The estimated source proportions therefore relate to these sampling points. Source estimates are scale dependent in that they can differ for different sampling locations along a channel network as the mixture of potential sources and their connectivity to the channel system varies spatially. Future work could extend the sediment sampling along the

channel systems of some of the catchments to improve the robustness of the estimates by reducing scale dependency. On the basis of catchment size, the improved consideration of scale dependency would be most relevant to the River South Tyne (~163 km^2), River Greta (~150 km^2), Rea Brook (~89 km^2), Arkle Beck (~67 km^2) and Afon Ystwyth (~60 km^2) catchments.

- Artificial mixtures were not used to assess the mixing model outputs.
- The source apportionment is for fine-grained sediment rather than sediment-associated heavy metal pollution. The two cannot necessarily be taken to represent the same thing since a source could be significant for sediment release whilst having low concentrations of sediment-associated contaminants, or vice versa. This issue has been explored for sediment-associated nutrient pollution in river systems.

6.3 Conclusions

Estimates of the contribution of mine waste to the fine-grained channel bed sediment ranged from 3% in the River Greta to 26% in Red Tarn Beck. High relative contributions were also predicted for the River East Allen (19%), Hayle (18%), Egglestone Beck (16%) and Bolingey Stream (16%). Higher contributions from mine waste were associated with steeper catchments, with good connectivity between mine waste facilities and the river, and where mine facilities comprised a larger proportion of the catchment.

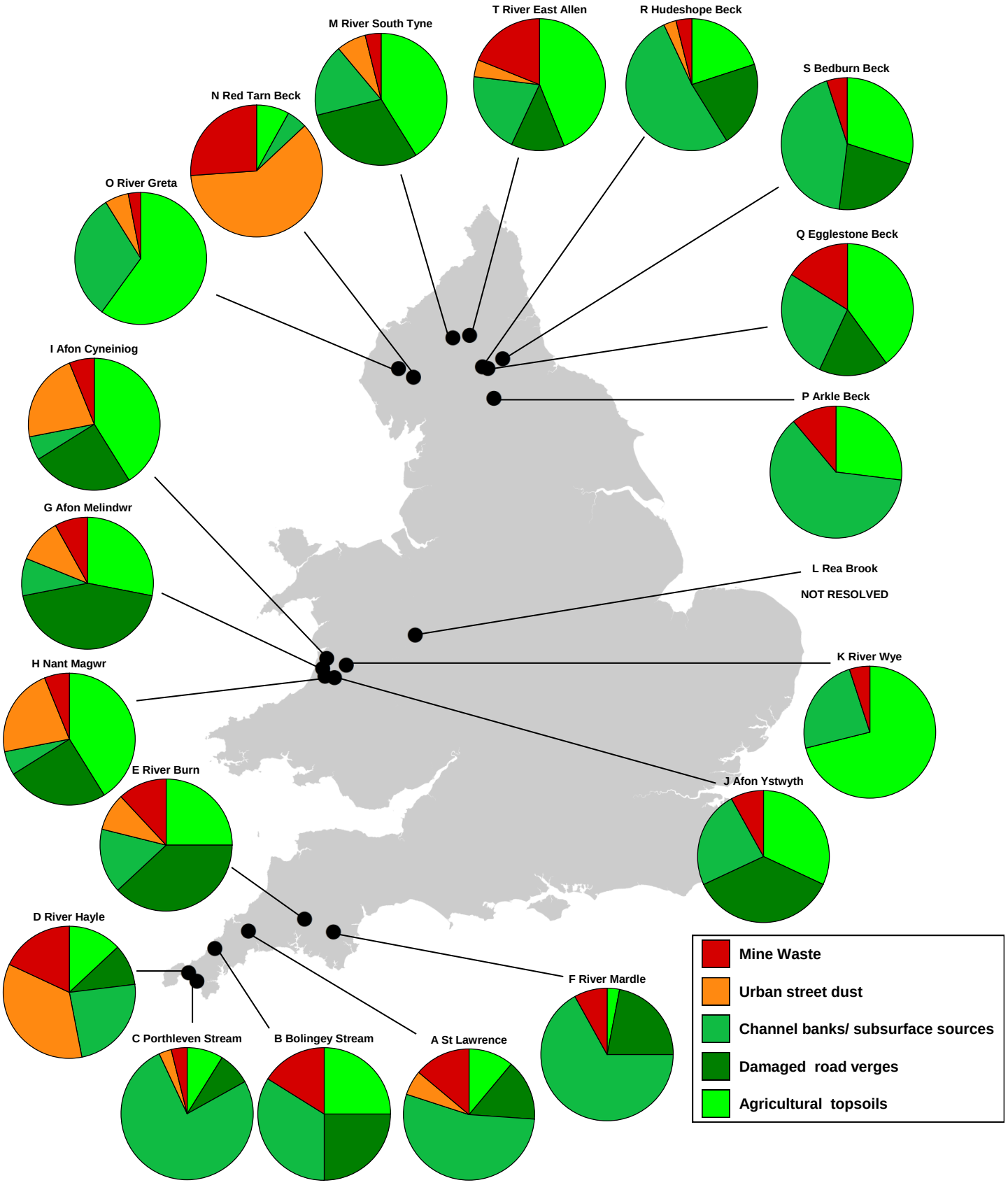
Table 6.16: Summary of estimated sediment source contributions in each study catchment, ranked by the proportion of mine waste.

Catchment	Code	Agricultural topsoils	Damaged road verges	Channel banks/ subsurface sources	Urban street dust	Mine Waste
Rea Brook	**L**	**NR**	**NR**	**NR**	**NR**	**NR**
River Greta	**O**	**0.6**	**-**	**0.31**	**0.06**	**0.03**
Porthleven Stream	**C**	**0.09**	**0.08**	**0.76**	**0.03**	**0.04**
River South Tyne	**M**	**0.41**	**0.3**	**0.18**	**0.07**	**0.04**
Hudeshope Beck	**R**	**0.2**	**0.21**	**0.52**	**0.03**	**0.04**
Wye	**K**	**0.71**	**-**	**0.24**	**-**	**0.05**
Bedburn Beck	**S**	**0.3**	**0.22**	**0.43**	**-**	**0.05**
Nant Magwr	**H**	**0.41**	**0.25**	**0.06**	**0.22**	**0.06**
Mardle	**F**	**0.03**	**0.22**	**0.67**	**-**	**0.08**
Afon Melindwr	**G**	**0.28**	**0.44**	**0.09**	**0.11**	**0.08**
Afon Cyneiniog	**I**	**0.86**	-	**0.06**	-	**0.08**
Afon Ystwyth	**J**	**0.32**	**0.36**	**0.24**	**-**	**0.08**
Arkle Beck	**P**	**0.27**	**-**	**0.62**	**-**	**0.11**
River Burn (Tavy)	**E**	**0.25**	**0.38**	**0.16**	**0.09**	**0.12**
St Lawrence Stream	**A**	**0.11**	**0.15**	**0.54**	**0.06**	**0.14**
Bolingey Stream	**B**	**0.25**	**0.25**	**0.34**	**-**	**0.16**
Egglestone Beck	**Q**	**0.4**	**0.17**	**0.27**	**-**	**0.16**
Hayle	**D**	**0.13**	**0.1**	**0.24**	**0.35**	**0.18**
River East Allen	**T**	**0.44**	**0.13**	**0.2**	**0.04**	**0.19**
Red Tarn Beck	**N**	**0.08**	**-**	**0.05**	**0.61**	**0.26**

NR – not resolved.

Figure 6.2 Summary of sediment source relative contributions in each study catchment.

Note that these estimates of weighted averages of relative (not absolute) contributions are subject to the limitations detailed in section 6.2.1.



7 Laboratory Experiments

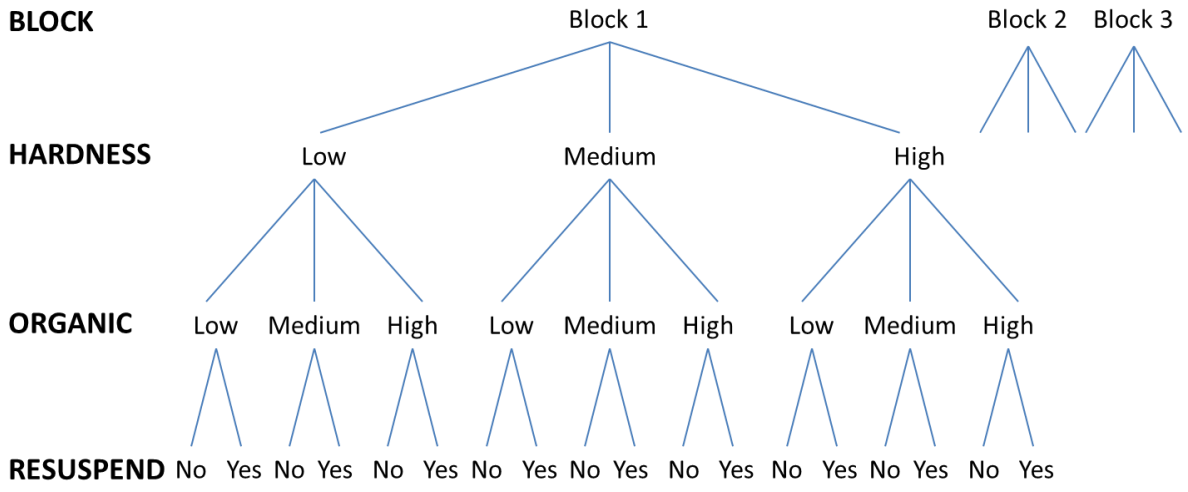
Objective 5a

Replicated laboratory mesocosm experiments (bioassays) will be conducted to quantify changes to metal partitioning in sediments (sampled from rivers across a gradient of contamination) and to understand the impacts on toxicity in response to altered environmental conditions (e.g. pH, redox potential; cf. Spencer et al 2006; Kadiri et al., 2012) and following cycles of sediment re-suspension and deposition. These experiments will further our understanding of how site-specific conditions alter the toxic effect and will inform the prioritisation of remediation works. Sediments and organisms will be analysed using the same protocols as WP3.

7.1 Methodology

A laboratory experiment was undertaken in replicated aquaria (mesocosms) where a chosen biomonitor species (*Baetis* spp) was incubated with contaminated sediment and overlying water under controlled conditions. The sediment was manipulated following a replicated block design, to provide experimental gradients of organic matter content of the sediment and water hardness, together with an additional treatment of resuspension/no resuspension (Figure 7.1). The body burden of metals in the biomonitor species was determined at the start (a sample of the *Baetis* spp to be used) and at the end of the incubation period, to establish the influence of the gradients of environmental conditions on the bioavailability of metals.

Figure 7.1 Schematic diagram of the experimental design used (total number of mesocosms = 54). All treatments were set up and allowed to equilibrate for three weeks. After this initial equilibration period the sediment in those replicates to be resuspended was disturbed and allowed to settle. The biomonitor species (*Beatis*) were then added and the experimental incubation period began.



Sediment collections for use in the laboratory experiment were made from three sites:

i) Brookwood mine settlement pond (river Mardle)
ii) The river Mardle downstream of Brookwood mine
iii) The downstream depositional site of Cotehele Stream

At each site, undisturbed areas with obvious deposits of fine sediment were located visually and collections of the bed made using a shovel and containers whilst ensuring minimal loss of fine material. On return to the laboratory, any overlying water was reduced by siphoning without drying the sediment. Samples of each type of sediment were collected for analysis of metal content using aqua regia extraction (see Section 4.2.4), which revealed a substantial metal content in all three sediments (Table 7.1; Figure 7.2).

It was decided to use the sediment from the River Mardle for the following reasons:

a) the material from the Brookwood Mine settlement pond was likely to be too toxic and lead to mortality of the biomonitor species.
b) the Cotehele stream was not included in the field survey, therefore there were no existing data available on the biomonitor species. In addition, for project consistency it was preferable not to use this site.
c) the metal content of the River Mardle sediment was sufficiently metal rich, particularly copper, to be used without mixing with the more contaminated settlement pond sediment.

Larvae of mayflies, *Baetis* spp., were chosen as the biomonitor species to be used as they are more abundant than caddis flies, making collection from the field easier, are robust to laboratory conditions, and had a high body burden of arsenic, cadmium, copper and zinc downstream of Brookwood mine at the Mardle site when collected during the field survey (Table 7.2). In addition, the *Baetis* spp. collected from the independent control used in the field survey (Dean Burn) had a low body burden of metals.

Water (conductivity approx. 40 µS) and *Baetis* to be used in the experiment were collected from Dean Burn, so that the only source of any metals accumulated by the *Baetis* was the sediment, either directly or indirectly.

Approximately 3 cm depth of sediment was added to each of the mesocosms.

To obtain organic matter, dried freshly fallen oak leaves collected from woodland at East Stoke, Dorset, were conditioned by incubating them in hessian sacks in the River Frome, Dorset, for 6 weeks. The conditioned leaf material was then rinsed clean of fine sediment and mashed with a blender. This material was mixed into the sediment once in the mesocosms. The mesocosms were then carefully filled with water and gently aerated.

The experimental treatments used in a fully factorial design were:

Organic matter: 0, 50 or 100 g wet weight of mashed leaf litter added to the sediment,
Water hardness: $CaCO_3$ added to the water to achieve 50, 150 or 250 mg l^{-1},
Resuspension: either mesocosom subject to a single resuspension event (inverted) or not.

The mesocosms were arranged in three blocks (Figure 7.3) and incubated at 12 °C for three weeks, for the sediment and water to equilibrate. After this initial equilibration period, a sample of the sediment was collected from each mesocosm and immediately frozen, for

determination of particle size distribution, organic carbon content, and Fe oxides (for methods see Section 4.2.4). These samples were also used to determine metal content, using aqua regia extraction (see Section 4.2.4), and by the BCR three step sequential extraction procedure as detailed in Rauret et al. (1999) using, step 1) acetic acid (0.11 mol l^{-1}), step 2) hydroxylamine hydrochloride (0.5 mol l^{-1}), and step 3) hydrogen peroxide (300 mg g^{-1}, which was evaporated off) and ammonium acetate (1.0 mol l^{-1}).

Table 7.1 Metal content of the three sediments collected for potential use in the experiments, together with ANZEEC & ARMCANZ and CCME sediment quality guidelines.

	Mean Concentrations (mg kg^{-1}). Range values shown in brackets							
	Co	**Cr**	**Cu**	**Mn**	**Ni**	**Pb**	**Sn**	**Zn**
Settlement Pond (*n=4*)	89 (24-124)	15 (15-17)	6048 (409-7082)	169 (169-195)	33 (33-41)	294 (29-325)	113 (13-174)	271 (141-305)
Mardle *n=5*	24 (21-27)	22 (20-27)	409 (158-1254)	689 (627-739)	36 (33-41)	28 (26-31)	102 (102-102)	140 (129-158)
Cotehele *n=5*	37 (30-48)	31 (28-40)	510 (395-608)	2323 (1236-5142)	68 (57-93)	69 (53-85)	12 (5-21)	461 (358-520)
ANZEEC & ARMCANZ Low Trigger		80	65		21	50		200
CCME ISQG		37.3	35.7			35		123
Suggested Theshold (Section 5.4)			34.3		39.8	133	9	447

Figure 7.2 Mean metal content (error bars show range) of the three sediments collected for potential use in the experiments

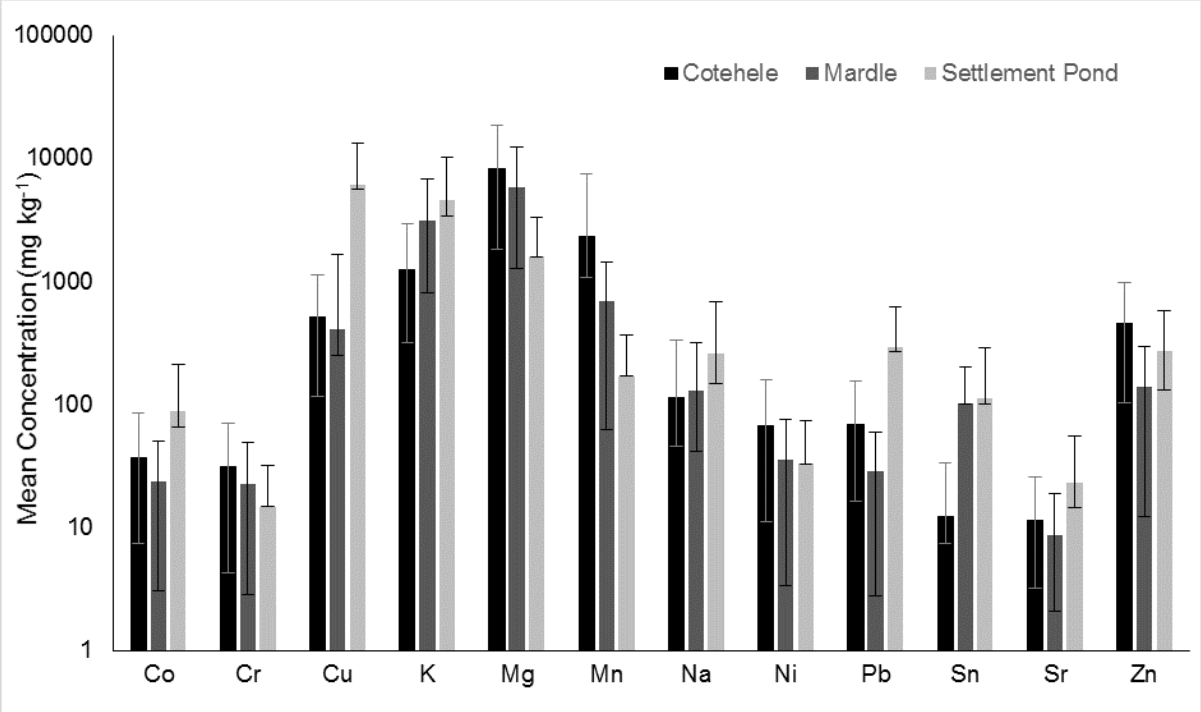


Table 7.2. Trace metal body burden (as µg g^{-1} dry weight) of *Baetis* spp. Collected from the River Mardle and Dean Burn (independent control) during the field survey (reproduced from Table 4.4). High local bioavailability of that metal for *Baetis* spp is indicated in red. Blank cells indicate metal below detection limits.

Site	As	Cd	Co	Cr	Cu	Mn	Ni	Pb	Zn
Upstream Control	6.5	3.24	4.32		35.1	127	22.8		218
Downstream	**127**	7.98	24.5		**111**	225	24.3	6.99	525
Downstream erosional	28.1	9.02	28.6	3.33	81.5	198	19.2	7.47	483
Downstream depositional	16.7	**19.0**	43.8	4.14	**128**	197	39.7	9.43	**1046**
Independent control	8.2	7.01	20.3	5.13	38.3	157	62.1	4.39	406

After the initial equilibration period, the sediment in those replicates to be resuspended was disturbed and allowed to settle. Then 20 individuals of the biomonitor species (*Baetis*) were added and the experimental incubation period began.

pH and dissolved oxygen of the water were measured through the initial equilibration period and incubation period with the *Baetis* present.

The *Baetis* were incubated in the mesocosms for two weeks. At the end of the incubation period the remaining *Baetis* individuals from each mesocosm were collected and immediately frozen. These *Baetis* were used to determine the trace metal body burden following the methods outlined in 4.2.2, thus acting as a measure of the bioavailability of metals under the experimental treatments. Once the collection of *Baetis* was completed a second sample of sediment was collected and immediately frozen, as before, for determination of particle size distribution, organic carbon content, and Fe oxides (for methods see Section 4.2.4). These samples were also used to determine metal content, using aqua regia extraction (see Section 4.2.4), and by the BCR three step sequential extraction procedure (Rauret et al., 1999).

Data were analysed using generalized linear models in SAS. A repeated measures ANOVA (for repeat samples collected over time) was used to determine the influence of the experimental treatments on the sediment and water. A MANOVA (for multiple measures collected at the same time) was used to establish the effect of the experimental treatments, and any interactions among them, on the body burden of trace metals in *Baetis*: our measure of the bioavailability of metals in the experimental mesocosms.

Figure 7.3 Mesocosms set up in the temperature controlled growth room.

7.2 Results

7.2.1 Water

The pH of the water varied significantly over the duration of the experiment (Table 7.3: Figure 7.4). pH also varied significantly with the addition of organic matter to the sediment, and there was an interaction between organic matter and time. An additional main effect of hardness was also apparent on the pH (Table 7.3), possibly due to increased buffering capacity in the treatments with larger additions of $CaCO_3$ (Figure 7.4). Dissolved oxygen was influenced by block (the aeration system was set up to reflect the block design) and time (Table 7.3), although the trend over time was not directional. There was also an effect of hardness, where the mesocosms that received larger additions of $CaCO_3$ had slightly, but significantly, higher concentrations of dissolved oxygen (Figure 7.4).

7.2.2 Sediment

As organic matter was added experimentally to the sediment there was a significant effect of this treatment on the percentage organic matter of the sediment collected from the mesocosms (Table 7.4: Figure 7.5a). The pH of the sediment was significantly affected by the organic matter additions (Table 7.4) where larger additions of organic matter resulted in a higher sediment pH (Figure 7.5b). There was also a significant effect of time, with a decrease in sediment pH between the first and second collection of sediment, presumably due to decomposition (Figure 7.5b). Oxalate extractable iron oxide increased between the two sediment collections, but was not significantly affected by the experimental treatments (Figure 7.5c). Dithionite extractable iron oxides did not vary significantly, hence the ratio of oxalate to dithionite extractable iron oxides increased significantly over time (Table 7.4).

There was no effect of treatment on the particle size distribution of the sediment. The sediments were mostly coarse, with the exception of a single replicate, silty sands (Figure 7.6).

Table 7.3 Results of repeated measures ANOVA on water pH and dissolved oxygen.

* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

	Water	
	pH	DO
Block		**
Organic	***	
Hardness	*	**
Resuspend		
Organic * Hardness		
Organic * Resuspend		
Hardness * Resuspend		
Organic * Hardness * Resuspend		
Time	***	***
Time * Block		***
Time * Organic	**	
Time * Hardness		
Time * Resuspend		

Figure 7.4 Variation of mean (± SE) pH and dissolved oxygen concentration of the water in the mesocosms over time and by main experimental treatments.

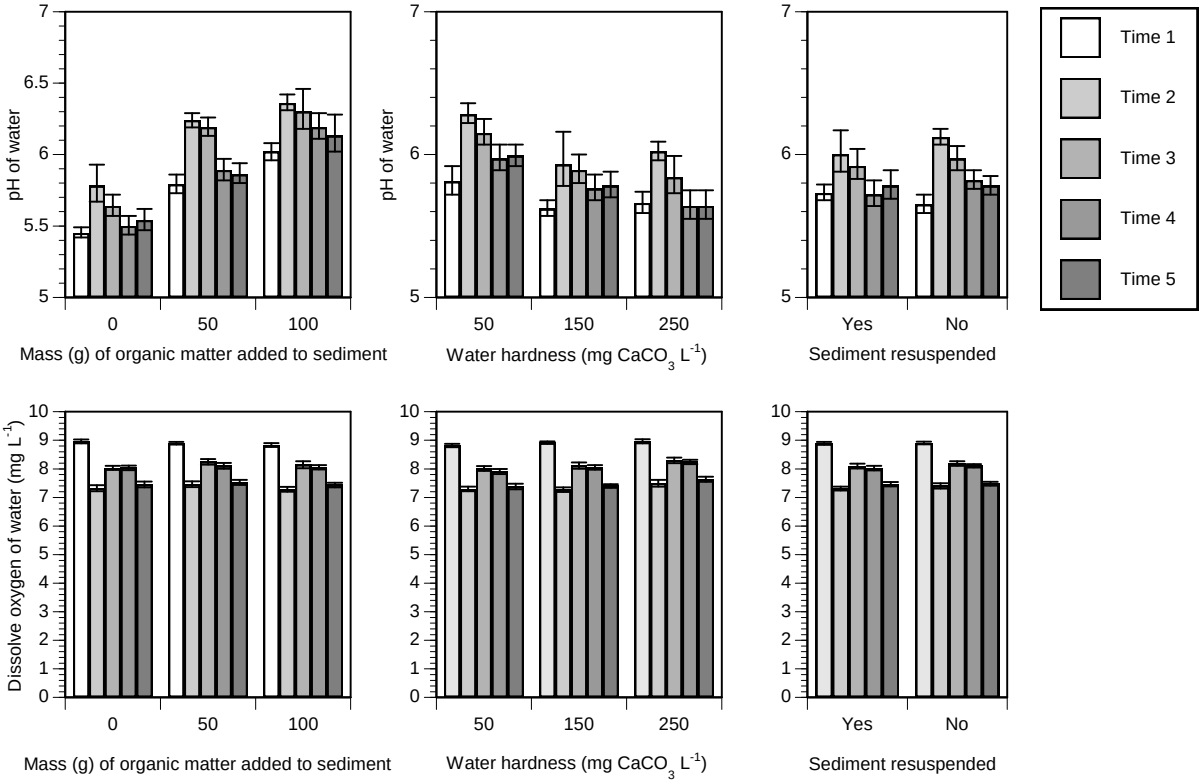


Table 7.4 Results of repeated measures ANOVA on sediment organic content, pH and iron oxides. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

	% Organic	pH	FeO Oxalate	FeO Dithionite	Oxalate: Dithionite
Block					
Organic	***	***			
Hardness					
Resuspend					
Organic * Hardness					
Organic * Resuspend					
Hardness * Resuspend					
Organic * Hardness * Resuspend					
Time		***	***		***
Time * Block					
Time * Organic					
Time * Hardness					
Time * Resuspend					

Figure 7.5 Variation in mean (± SE) % organic content, pH and oxlate extractable iron oxide concentration in the sediment over time and by main experimental treatments.

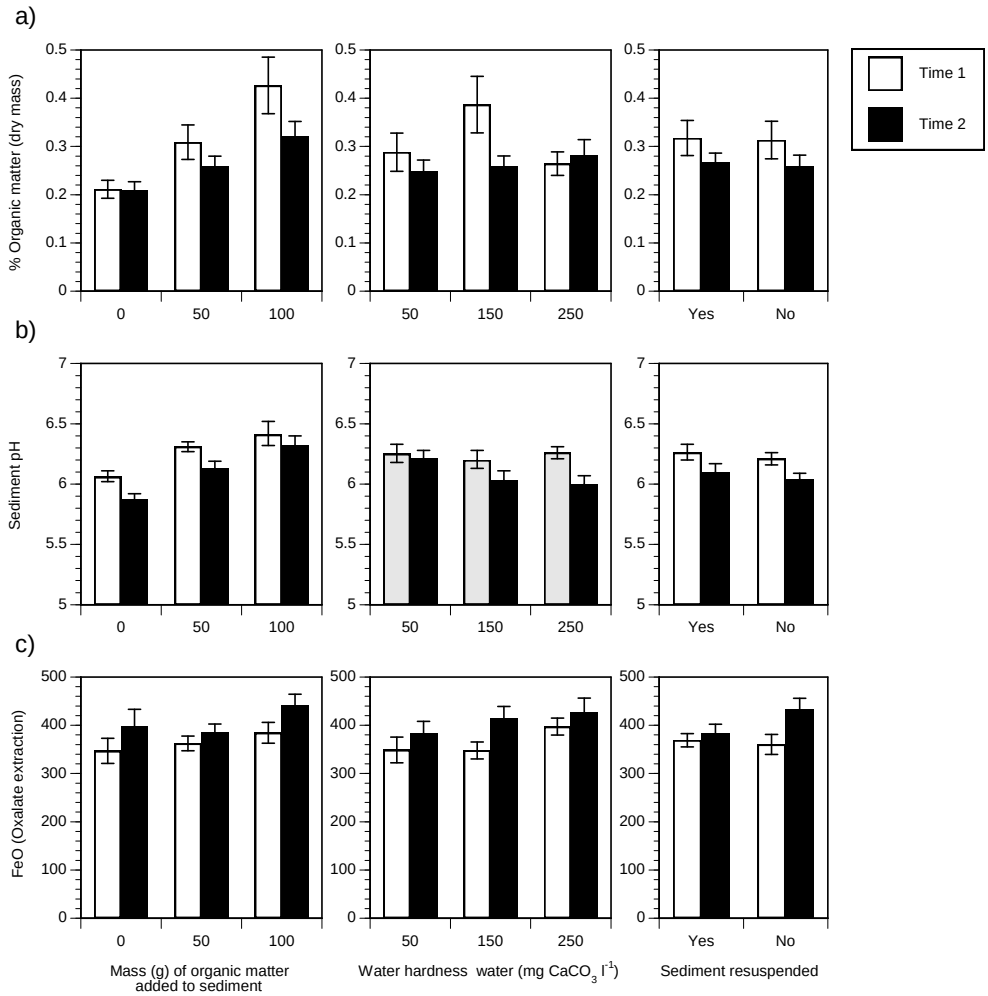
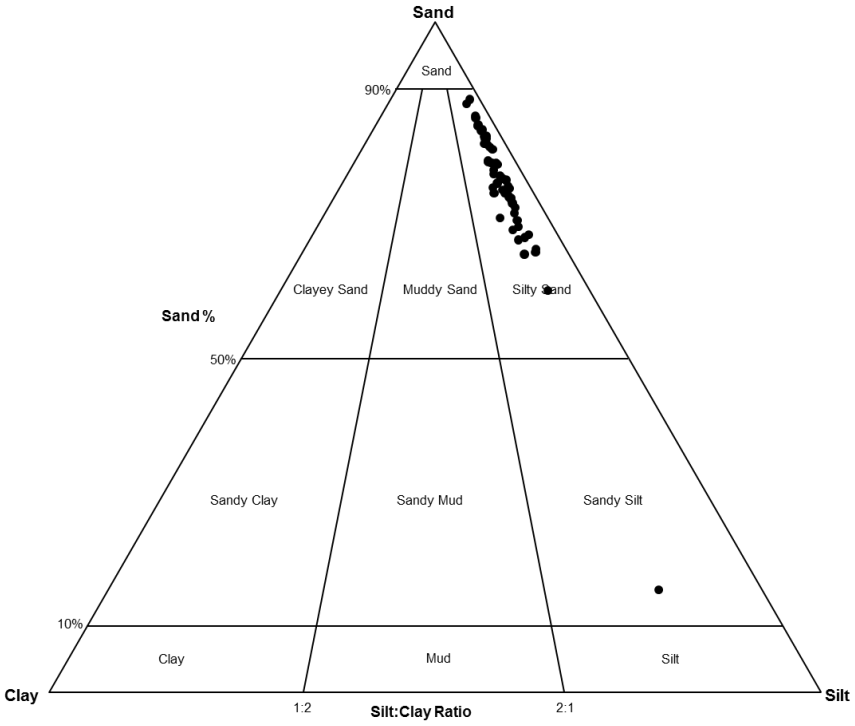


Figure 7.6 Ternary diagram of particle size composition of the sediment collected at the end of the experiment.



Aqua regia extraction

The aqua regia extractable metal content of the sediment varied significantly over time (MANOVA $p \leq 0.0001$), with a tendency for concentrations to decrease between the first and second sampling occasions, particularly cadmium, copper, iron and lead (Figure 7.7: Table 7.5). There was also an interaction between organic matter and time for aqua regia extractable lead, where a more pronounced decrease over time was seen in treatments with more organic matter (Figure 7.7e). Resuspension increased the aqua regia extractable concentration of nickel (Table 7.5), and there was an interaction between organic matter and resuspension on the aqua regia extractable lead concentration (Table 7.5), where higher concentrations were observed in resuspended treatments with more organic matter.

BCR three step sequential extraction

As sequential extraction provides information on the partitioning of metals in the sediment, results are presented for both the metals of interest and other metals.

The method of extraction had a highly significant effect on all metals except lead, where the effect was close to significant (Table 7.6). The concentrations of lead were low with all three extraction methods. Concentrations of barium and zinc decreased with increasingly aggressive extraction method suggesting that these metals were largely readily exchangeable, soluble in either water or acid (i.e. as carbonates), or in oxidised forms (Figures 7.8-7.11a & j). The highest concentrations of iron and manganese were obtained with step 2, hydroxylamine hydrochloride, suggesting that these metals were mainly present in oxidised forms (Figures 7.8-7.11e & h). Concentrations of calcium and magnesium were lowest when extracted with hydroxylamine hydrochloride and highest when extracted with hydrogen peroxide and ammonium acetate suggesting they were mostly either present in reduced forms, associated with organic matter, or readily exchangeable, possibly as carbonates or associated with clay minerals (Figures 7.8-7.11b & g). Chromium, copper, potassium and lead increased with increasingly aggressive extraction method, suggesting that these metals were present in the oxidisable fraction, either in a reduced form or associated with organic matter (Figures 7.8-7.11c, d, f, g & i). Cadmium and nickel were not detected with any of the three sequential extractions, but were extracted with aqua regia (Figure 7.7), suggesting they were largely present in recalcitrant, possibly mineral, forms.

Time had an influence on measured concentrations of chromium, iron and (close to significant) lead (Table 7.6) where, as with the aqua regia extractions, measured concentrations were lower on the second occasion (Figure 7.8). There was also a close to significant interaction between time and extraction method for chromium, iron and zinc, but these largely reflected the main effect of time; where the metals were detected they tended to decrease over time. However, the significant interaction between time and extraction method for copper (Table 7.6) reflected a different response: concentrations measured with hydrogen peroxide and ammonium acetate increased with time, but declined for acetic acid and hydroxylamine hydrochloride, suggesting that copper became more associated with organic matter, and hence less mobile, over time.

The organic matter added experimentally to the sediment only had a significant influence on barium (Figure 7.9: Table 7.6), where measured concentrations were highest in sediments that received the largest amounts of organic matter. There was a significant interaction

between organic matter and extraction method for calcium (Table 7.6): measured concentrations increased with organic matter additions when extracted with acetic acid, but decreased with organic matter additions when extracted with hydrogen peroxide and ammonium acetate (Figure 7.9). There was a close to significant interaction between time and organic matter for barium and manganese. Magnesium also returned a significant interaction between organic matter, extraction method, hardness and resuspension, and a close to significant interaction between organic matter, hardness and resuspension. Organic matter additions to the sediment had no significant effect on any other metal, irrespective of extraction method.

Water hardness had a significant effect on calcium, copper, iron and magnesium, and a significant interaction with extraction method for calcium, copper, potassium and magnesium (Table 7.6). Measured concentrations of these metals were higher with increased hardness, particularly when hydrogen peroxide and ammonium acetate were used (Figure 7.10), suggesting that hardness influenced the oxidisable fraction, i.e. either in a reduced form or associated with organic matter. However, for calcium, increased hardness also influenced measured concentrations when acetic acid was used for extraction (Figure 7.10b), suggesting precipitation as carbonate.

Resuspension had a significant influence on calcium, iron, magnesium and zinc, and an interaction with extraction method for the same metals and lead (Table 7.6). Resuspension tended to result in lower measured concentrations particularly when hydrogen peroxide and ammonium acetate were used for extraction, with the exception of lead where this fraction increased with resuspension (Figure 7.11).

To better understand how metals were responding to the experimental treatments, metals were grouped following their response to the BCR three step sequential extraction, i.e. according to their partitioning within the sediments, and the data analysed by these groups using MANOVA. The four groups were:

1. Metals that were mainly in readily exchangeable forms, soluble in either water or acid (i.e. as carbonates), where concentrations were highest when extracted with acetic acid, comprising barium, strontium and zinc.
2. Metals that were mainly present in oxidised forms, where concentrations were highest when extracted with hydroxylamine hydrochloride, comprising cobalt, iron and manganese.
3. Metals that were mainly present in the oxidisable fraction, either in a reduced form or associated with organic matter, where concentrations were highest when extracted with hydrogen peroxide and ammonium acetate, comprising silver, aluminium, chromium, copper, potassium and lead.
4. Metals that were mainly in either readily exchangeable or reduced forms, where concentrations were lowest when extracted with hydroxylamine hydrochloride, comprising calcium, lithium, magnesium and sodium.

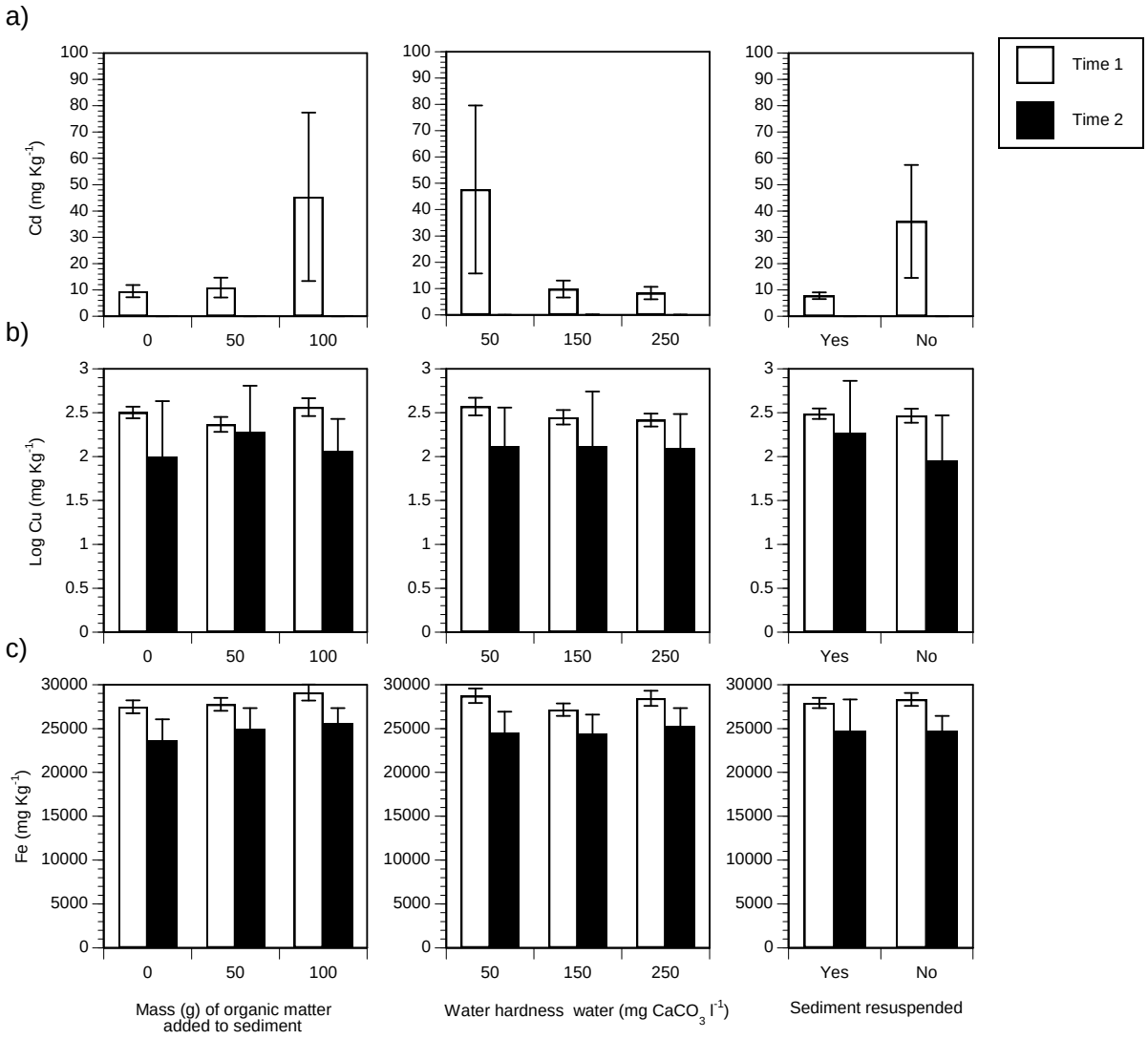
Where detected, measured concentrations of readily exchangeable metals (Group 1) in the sediment were significantly lower with time (time*extraction), suggesting release to the water column, which was significantly enhanced by resuspension (Table 7.7).

Measured concentrations of metals that were mainly present in oxidised forms (Group 2), were influenced by time, water hardness and resuspension, suggesting loss to the water column particularly where the water was soft or the sediment disturbed (Table 7.7).

Measured concentrations of metals that were mainly present in reduced forms (Group 3), were also influenced by time, water hardness (both close to significant) and resuspension, suggesting some loss to the water column particularly where the water was soft (Table 7.7). However, here resuspension tended to result in higher measured concentrations, suggesting release from mineral forms when the sediment was disturbed.

Metals that were mainly in either readily exchangeable or reduced forms (Group 4), were close to significant for the interaction between time and extraction method, becoming more reduced over time, i.e. moving towards more strongly held forms over time and becoming less labile, and significant for the interaction between organic matter and extraction method (Table 7.7), where increased organic matter was associated with an increase in readily exchangeable forms. Here also, resuspension and the interaction between resuspension and extraction method were significant, indicating release from the forms present when the sediment was disturbed. The interactions between organic matter and resuspension, extraction method, water hardness and resuspension, and organic matter, water hardness and resuspension were close to significant for these metals also (Table 7.7), suggesting that these metals were more influenced by environmental conditions than the other groups.

Figure 7.7 Variation in mean (± SE) aqua regia extractable metal concentration in the sediment over time and by main experimental treatments, a) Cadmium, b) Copper, c) Iron, d) Nickel, e) Lead and f) Zinc.



(Continued overleaf)

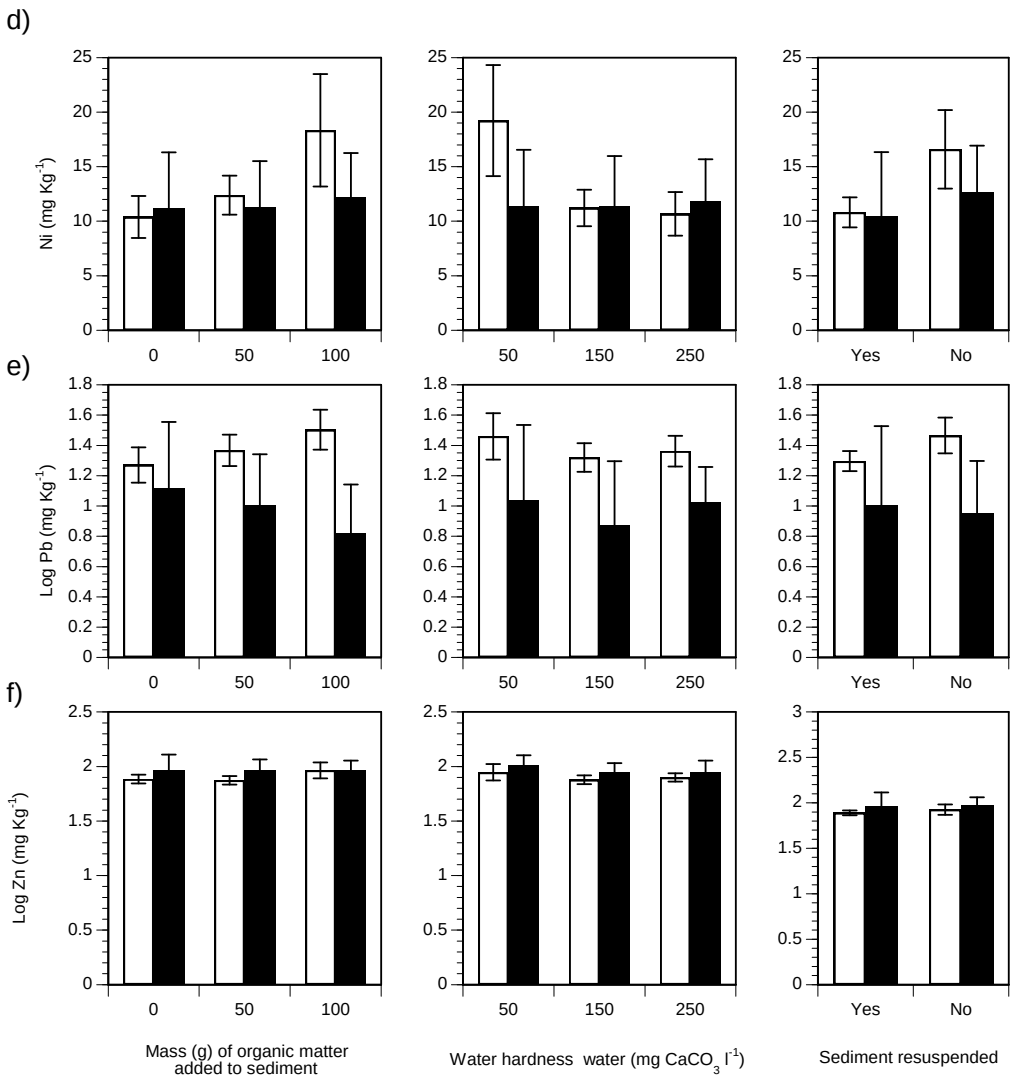


Table 7.5 Results of repeated measures ANOVA on aqua regia extractable sediment metal content.

	Sediment					
	Cd	Cu	Fe	Ni	Pb	Zn
Block						
Organic						
Hardness						
Resuspend				*		
Organic * Hardness						
Organic * Resuspend					**	
Hardness * Resuspend						
Organic * Hardness * Resuspend						
Time	***	***	***		***	
Time * Block						
Time * Organic					**	
Time * Hardness						
Time * Resuspend						

** P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001.*

Table 7.6 Results of ANOVA on sediment metal content determined by sequential extractions.

	Ca	Ba	Cr	Cu	Fe	K	Mg	Mn	Pb	Zn
Block										
Extraction	***	***	***	***	***	***	***	***	‡	***
Time			*		*				‡	
Time*Extraction			‡	*	‡					‡
Org		*								
Org*Extraction	***									
Time*Org		‡						‡		
Time*Org*Extraction										
Hard	***			**	**		*			
Extraction*Hard	**			**		*	**			
Time*Hard									‡	
Time*Extraction*Hard										
Org*Hard							‡			
Org*Extraction*Hard							**			
Time*Org*Hard				*						
Time*Org*Extrac*Hard										
Resusp	***				***		***			*
Extraction*Resusp	**				**		***		**	‡
Time*Resusp								‡		
Time*Extracti*Resusp								*		
Org*Resusp	*									
Org*Extractio*Resusp										
Time*Org*Resusp										
Time*Org*Extra*Resus								*		
Hard*Resusp										
Extracti*Hard*Resusp										
Time*Hard*Resusp										
Time*Extr*Hard*Resus			*							
Org*Hard*Resusp							‡			
Org*Extra*Hard*Resusp							*			
Time*Org*Hard*Resusp										
Tim*Org*Ext*Har*Resu										

‡ *P ≤ 0.01, * P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001.*

Figure 7.8 Influence of time on mean (± SE) metal concentrations determined by sequential extraction of the sediment, a) Barium, b) Calcium, c) Chromium, d) Copper, e) Iron, f) Potassium, g) Magnesium, h) Manganese, i) Lead and j) Zinc.

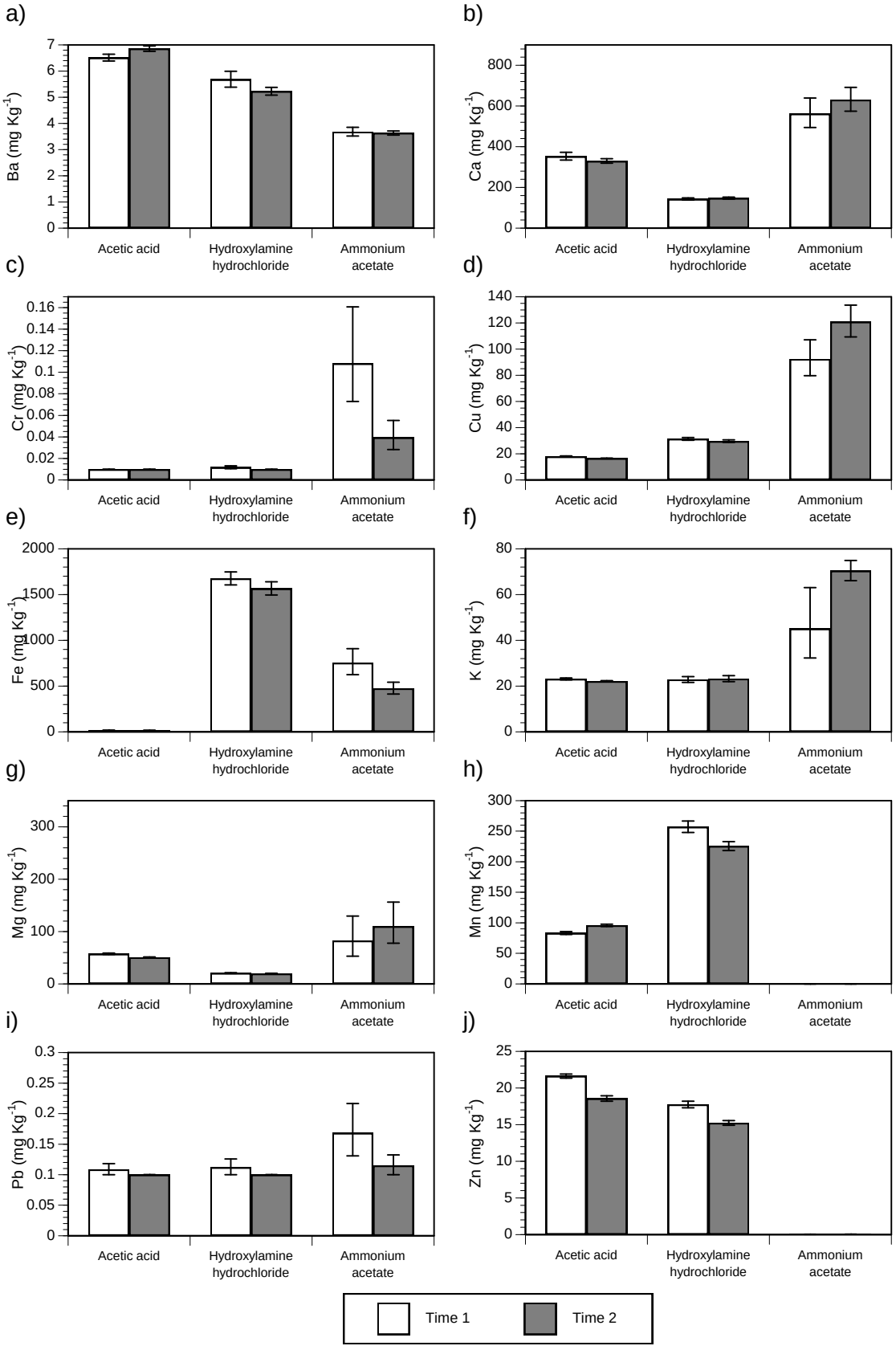


Figure 7.9 Influence of sediment organic matter on mean (± SE) metal concentrations determined by sequential extraction, a) Barium, b) Calcium, c) Chromium, d) Copper, e) Iron, f) Potassium, g) Magnesium, h) Manganese, i) Lead and j) Zinc.

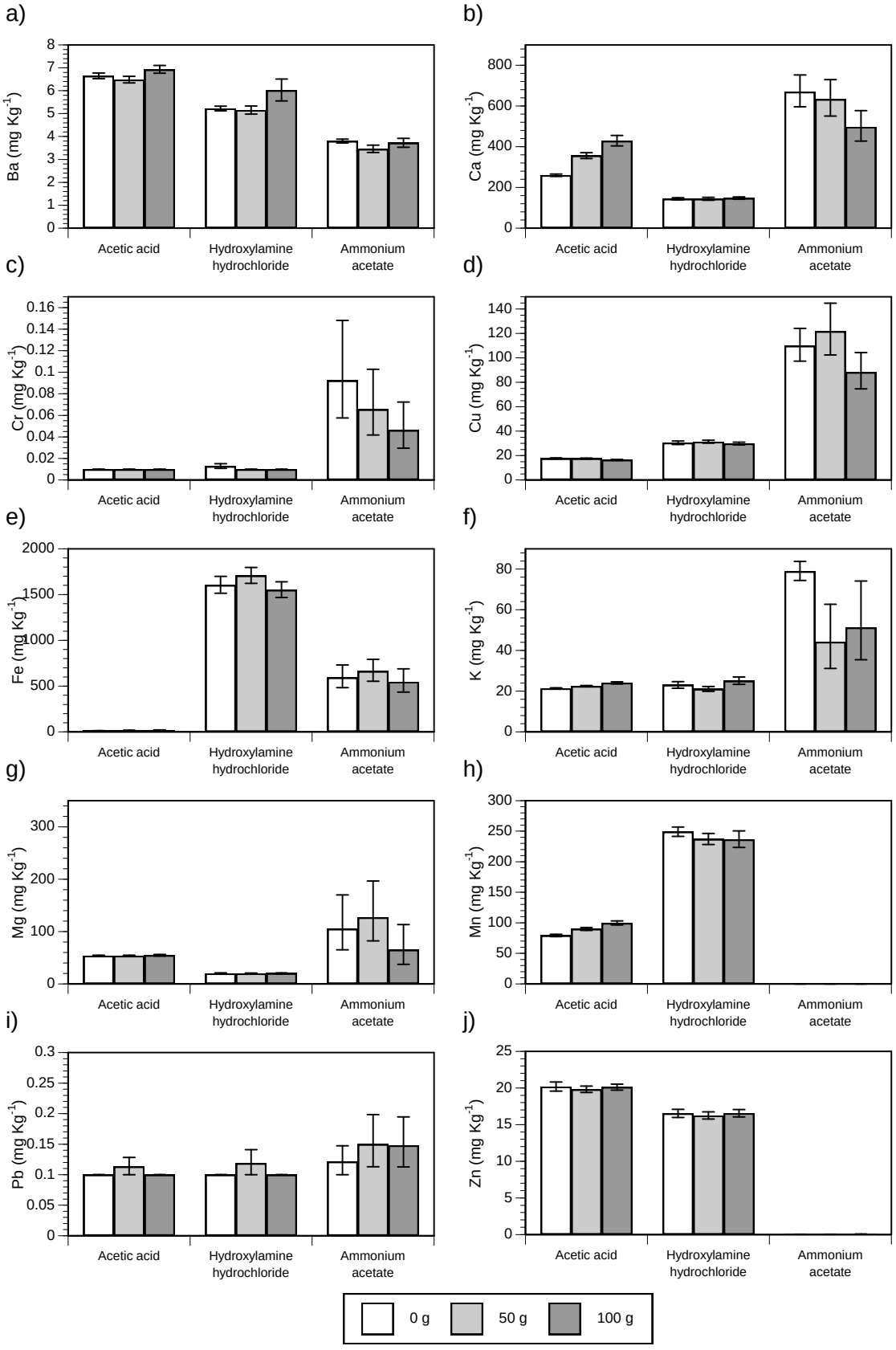


Figure 7.10 Influence of water hardness on mean (± SE) metal concentrations determined by sequential extraction, a) Barium, b) Calcium, c) Chromium, d) Copper, e) Iron, f) Potassium, g) Magnesium, h) Manganese, i) Lead and j) Zinc.

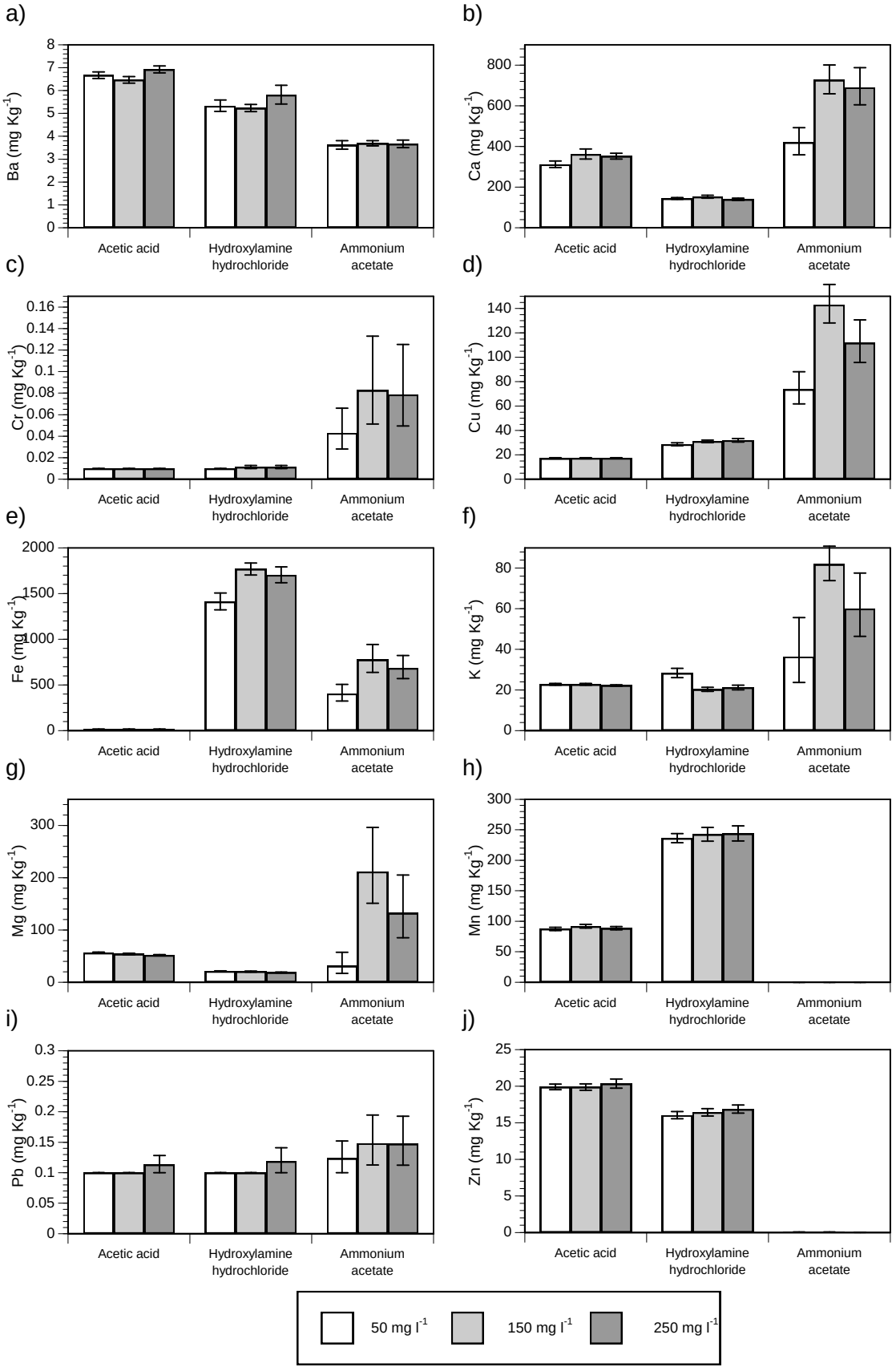


Figure 7.11 Influence resuspension of sediment on mean (± SE) metal concentrations determined by sequential extraction, a) Barium, b) Calcium, c) Chromium, d) Copper, e) Iron, f) Potassium, g) Magnesium, h) Manganese, i) Lead and j) Zinc.

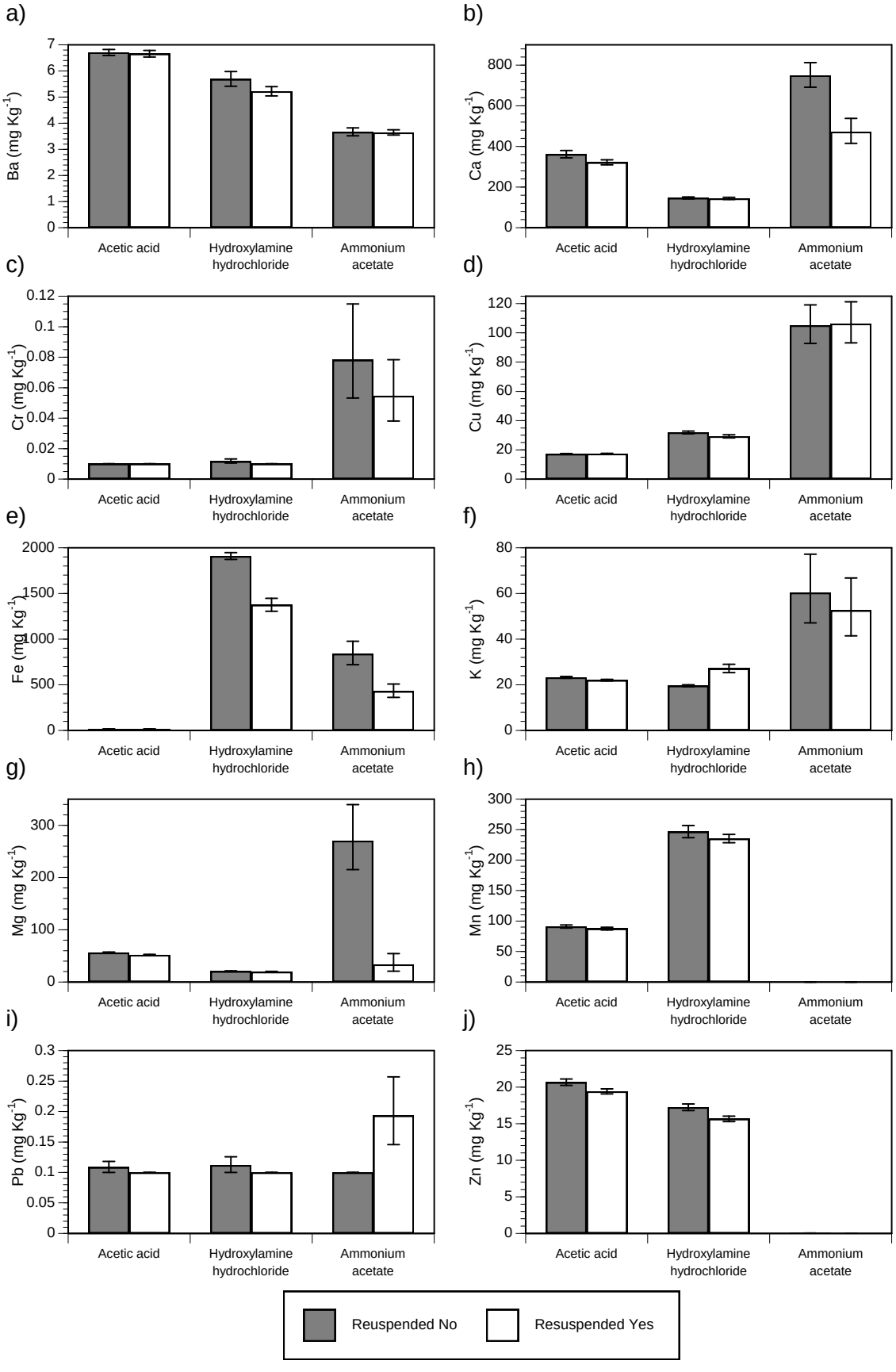


Table 7.7 Results of MANOVA on sediment metal content in groups determined by their response to sequential extractions (see text for details).

	Grp 1	Grp 2	Grp 3	Grp 4
	Ba, Sr, Zn	Co, Fe, Mn	Ag, Al, Cr, Cu, K, Pb	Ca, Li, Mg, Na
Block				
Extraction	***	***	***	***
Time		*	‡	
Time*Extraction	*	‡		‡
Org				
Org*Extraction				***
Time*Org				
Time*Org*Extraction				
Hard		*	‡	*
Extraction*Hard			‡	*
Time*Hard				
Time*Extraction*Hard				
Org*Hard				
Org*Extraction*Hard				‡
Time*Org*Hard				
Time*Org*Extrac*Hard				
Resusp	**	***	***	***
Extraction*Resusp		*	***	***
Time*Resusp				
Time*Extracti*Resusp				
Org*Resusp				‡
Org*Extractio*Resusp				
Time*Org*Resusp				
Time*Org*Extra*Resus		‡		
Hard*Resusp				
Extracti*Hard*Resusp				‡
Time*Hard*Resusp				
Time*Extr*Hard*Resus				
Org*Hard*Resusp				‡
Org*Extra*Hard*Resus				
Time*Org*Hard*Resusp				
Tim*Org*Ext*Har*Resu				

‡ *P ≤ 0.01, * P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001.*

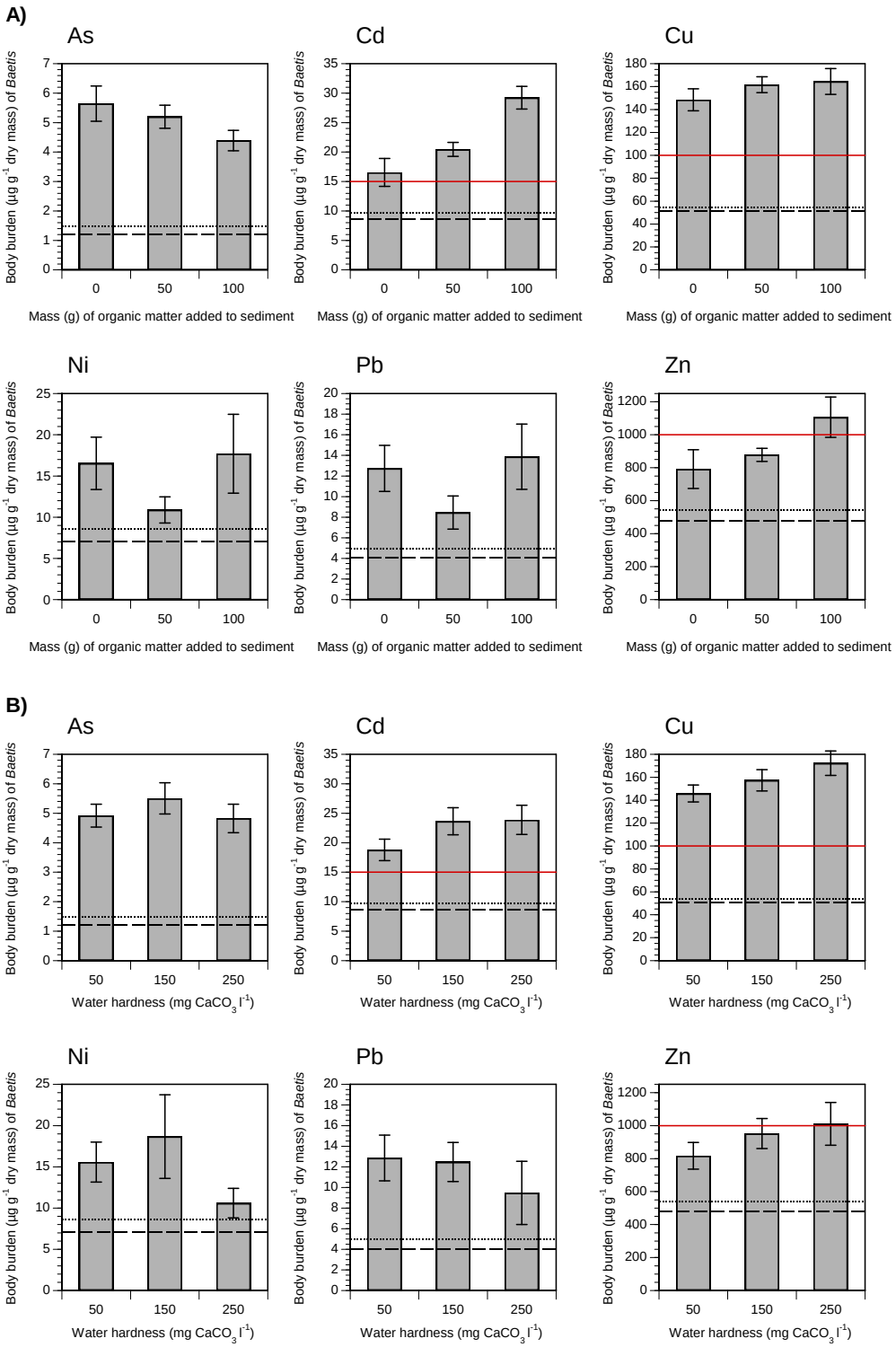
7.2.3 Bioavailability

The body burden of all metals in *Baetis* increased over the duration of the incubation, compared with the starting condition (Figure 7.12), particularly those metals that were in notably high concentrations in the sediment and *Baetis* collected from the river Mardle (i.e. arsenic, cadmium, copper and zinc). However, it should be noted that only cadmium, copper and zinc were found in the tissues of *Baetis* in concentrations that would be considered high (i.e. >15, >100 and >1,000 µg g^{-1} respectively: see Section 4.3.1 and Table 4.4). In addition there was a significant effect of the organic matter added to the sediment: body burdens, particularly of cadmium, copper and zinc, were higher in *Baetis* from those treatments that had larger amounts of organic matter added to the sediment (Table 7.7: Figure 7.12a). On the other hand, the body burden of arsenic tended to be lower with increased organic matter in the sediment. MANOVA detected marginally significant effects of water hardness (p = 0.0575) and the interaction between water hardness and resuspension (p = 0.0933): in contrast with the effect of hardness in field studies, bioavailability was marginally higher in treatments with increased $CaCO_3$ (Figure 7.12b).

Table 7.6 Results of MANOVA on body burden of arsenic, cadmium, copper, nickel, lead and zinc in *Baetis* at the end of the incubation period.

	p
Block	
Organic	0.0018
Hardness	0.0575
Resuspend	
Organic * Hardness	
Organic * Resuspend	
Hardness * Resuspend	0.0933
Organic * Hardness * Resuspend	

Figure 7.12 Variation in body burden (mean ± SE) of arsenic, cadmium, copper, nickel, lead and zinc in *Baetis* in response to A) organic matter and B) water hardness.
Horizontal dashed line = body burden at the start of the experiment (+ SE dotted line); horizontal red line = body burden considered high for *Baetis* (As and Pb = 100 µg mg^{-1}).



7.3 Discussion

The sediment collected from the River Mardle was contaminated with metals from the Brookwood Mine. In particular, copper and nickel concentrations were in excess of the ANZEEC & ARMCANZ Low Trigger concentration and zinc concentrations were in excess of the CCME ISQG. Furthermore, the *Baetis* collected from the Mardle during the field survey had high body burdens of arsenic, cadmium, copper and zinc, indicating that bioavailability of these metals was high in the River Mardle. The body burden of all metals in *Baetis* increased over the duration of the incubation, compared with the starting condition. At the end of the experiment the body burdens of cadmium, copper and zinc were in excess of, or close to concentrations considered high for *Baetis* (15, 100 and 1,000 µg g^{-1} respectively: see Section 4.3.1 and Table 4.4). As both the water and *Baetis* used in the experiment were from the Dean Burn, the sediment was the only substantial source of metals. It is apparent that the contaminated sediment from the River Mardle was acting as a source of bioavailable metals in this experiment. From this we can conclude that it is likely, even where mine drainage water is treated to reduce metal concentrations, contaminated sediments, including river bed sediment, will act as a source of bioavailable metals.

Furthermore, the addition of organic matter to the sediment increased bioavailability significantly, particularly of cadmium, copper and zinc. The bioavailability of arsenic tended to decrease with organic matter additions perhaps indicative of arsenic partitioning in the sediment. However, it should be noted that the body burdens of arsenic were not high (<10 µg g^{-1} *cf.* >100 µg g^{-1}: see Section 4.3.1 and Table 4). Additions of organic matter were also associated with increased pH of the sediment and water, but did not appear to have a substantial influence on the concentrations of metals extracted from the sediment using either aqua regia or the BCR three step sequential extraction method (Tables 7.5, 7.6 & 7.7). This latter result is somewhat surprising: the addition of particulate organic matter to the sediment did not have a substantial effect on the partitioning of metals in the sediment, but did influence bioavailability, suggesting that bioavailability was influenced by more than just the form that the metals were in. As *Baetis* consume particulate organic matter it is possible that they were accessing metals via their diet.

The BCR three step sequential extractions of sediment indicated that zinc was relatively readily available to solution from the sediment and was not associated with organic matter in the sediment. In contrast, copper, lead and cadmium were all relatively recalcitrant, with copper and lead mainly in the oxidisable fraction, either in a reduced form or associated with organic matter. Cadmium was only retrieved in measurable concentrations when extracted with aqua regia.

Dissolved organic carbon (DOC) reduces the bioavailability of metals in solution by forming complexes with the metals. It is likely that the mashed leaves added to the sediment did release DOC into the water, particularly as the organic matter appeared to decompose over time. It has been noted previously that increased DOC (along with higher water ionic concentration) can encourage metals into solution (Butler, 2009), but such an effect is most likely as a result of DOC (and the other factors) forming complexes with metals from the sediment. Here we used particulate organic matter and it is probable that in addition to releasing DOC, metals present in the oxidisable fraction (Ag, Al, Cr, Cu, K and Pb) were associated with this particulate material, and increasingly so as time progressed. Such

particulate material would in turn provide a route for uptake by the *Baetis* biomonitors, which consume fine particulate material.

It is not clear how the organic matter additions influenced the bioavailability of zinc, which appeared to be mainly in a readily exchangeable form in the sediment, suggesting that it was not strongly associated with any of the sediment fractions.

Water hardness had a marginally significant effect on bioavailability, with increased hardness associated with increased uptake of metals. This again appears contrary to expectations: water hardness reduces bioavailability of dissolved metals (albeit with less effect than DOC), through precipitation of insoluble salts. This was seen to a certain extent where metals mainly present in oxidised (Group 2: Co, Fe, Mn), reduced (Group 3: Ag, Al, Cr, Cu, K, Pb) and either reduced or readily exchangeable forms (Group 4: Ca, Li, Mg, Na) were found in the sediment at lower concentrations in the softer water. Whilst increased ionic activity of water does encourage metals out of sediment (Butler, 2009), and it is possible that any precipitates could have been ingested by the *Baetis*, too much weight should not be put on the influence of hardness as the effect was only marginally significant.

The effect of the single resuspension event did not cause any increase in bioavailability, yet it did cause effects on several of the metals. The increased exposure of deeper sediment, and increased physical mixing of water and sediment, that occurred as a consequence of such an event appeared to alter partitioning of the metals in the sediment and exchange with the water column. It had been presumed that such changes would influence bioavailability, but this did not appear to be the case. Again, it appears that bioavailability was influenced by more than the behaviour of the metals. Whilst, this is an encouraging result, suggesting that disturbance of this sediment through events such as flooding is unlikely to influence bioavailability, only a single resuspension was undertaken and (despite the fine sediment component being collected from the river) the sediment used was relatively coarse. It is possible that disturbance of other, finer sediments would have a more substantial impact and that the changes in metal partitioning and with the water column may influence bioavailability in the longer term.

Overall, the disparity between the influence of the experimental treatments on bioavailability and on the behaviour of metals in the sediment suggests that the uptake of metals by biota is influenced by some factor other than the chemical behaviour of the metals. We suggest that this is most likely to be biological, i.e. the consumption of metals through the diet.

8 Simple Rules to Identify Where Contaminated Sediments Pose Greatest Risk

Objective 6a. To develop simple rules and build upon existing models for characterising the effect on ecology of release or remobilisation of contaminants from sediment and to identify when remediation of sediment is required in addition to treatment of mine water discharge.

We have attempted to identify a series of criteria that will help identify whether a site is likely to be at risk from metals associated with sediments. The risk from contaminated sediment from abandoned metal mine facilities is comprised of four components:

A) the concentration of contaminants in the source material,
B) the delivery of contaminated sediment to the site,
C) the retention of contaminated sediment at the site,
D) the influence of environmental conditions at the site on bioavailability of metals from contaminated sediment.

These four components can be used to develop simple rules to characterise the effect of contaminated sediment on ecology.

8.1 Concentration of contaminants in the source material

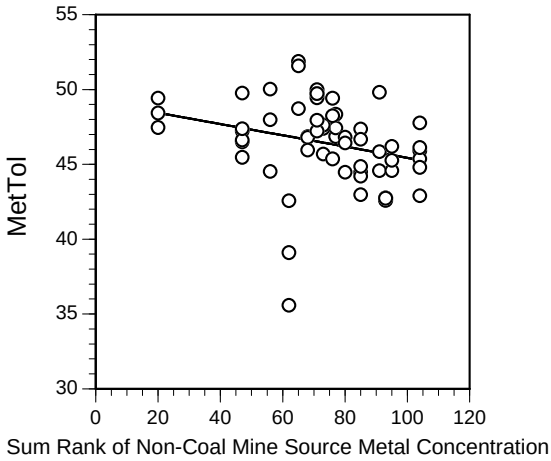
The data describing sediment sources from the apportionment work (Section 6) was used to assess the influence of the concentration of metals in the source material at abandoned metal mining facilities. To account for differences in absolute concentrations among metals, the mean metal concentrations in metal mine sediment sources were ranked, 1 to 20, for each metal (arsenic, cadmium, copper, nickel, lead, tin and zinc), with the highest rank ascribed to the catchment with the highest concentration of that metal. The sum of all ranks was then calculated for each catchment as a measure of the relative contamination of source material, with a maximum value of 140. Actual values ranged from 20 (Catchment T, River East Allen) to 104 (Catchments I, Afon Cyneiniog, and M, South Tyne). To determine the relationship between the concentration of contaminants in source material and ecological damage, the sum of ranks was then compared with the MetTol index value for the sites downstream of the abandoned non-coal mining facilities in each catchment.

A significant negative relationship was found between ecological damage and relative contamination of source material (Figure 8.1), indicating that the risk was greater at sites where the sources of metal mine sediment were more contaminated with metals. Hence, we suggest the following influence,

1) *The concentration of metals in the source material*

The extent of contamination of metal mine sediment sources at a site will depend on a variety of factors including the geology (of both mineral veins and overburden), economics (value of minerals extracted or discarded) and location (activities undertaken on site, e.g. crushing or smelting). There does not appear to be a strong pattern in the extent of source contamination associated with mine types or regions.

Figure 8.1 Relationship between relative contamination of non-coal mine source material and ecological damage, as MetTol index value. *p* = 0.036



8.2 Delivery of contaminated sediment to the site

The risk from contaminated metal mine sediment sources is dependent upon the sediment entering the river and being retained at a site. Here we have explored the extent to which metal mine sediment sources contribute to the biologically active fine component of river bed sediments using source apportionment approaches, and have provided estimates of the relative importance of metal mine sources in the 20 catchments studied. Using these results, and our understanding of sediment source delivery we have derived the following influences associated with risk.

2) *Grain-size of the mine workings/waste.*
3) *Proximity of metal mine sediment sources to flowing watercourses.*
4) *Gradient of catchment containing metal mine sediment sources.*
5) *Indications of erosion of metal mine sediment sources.*
6) *Surface vegetation covering metal mine sediment sources.*
7) *Effective pollution mitigation measures including settling ponds.*

The catchment where metal mine sources contributed most was N, Red Tarn Beck, and the least was O, River Glendermackin/River Greta. The former is a steep upland catchment where the mine is next to the river, a tributary flows through the mine, and the catchment is relatively small. In the latter, the mine is on a small tributary (Gategill Beck) within a large catchment (~150 km^2): whilst the mine has a significant impact upon Gategill Beck, the contribution to the sediment in the main river is small (3%).

8) *Footprint of metal mine sites within the catchment, i.e. the proportion of the catchment that the mine facility comprises.*

In catchments where the metal sources comprise a small proportion of the sediment relative to uncontaminated sources, the concentration of metals in river bed sediment is diluted: the results presented in Sections 4.3.4 and 4.3.5 indicate that the bioavailability of metals from sediment is related to concentration in the sediment rather than total load (concentration x mass of fine sediment). Hence, we suggest the following influence,

9) *Sediment from other, uncontaminated sources.*

The source apportionment work indicated that sediment from eroding agricultural topsoils comprised a smaller proportion where the catchment was dominated by rough grazing, particularly catchment F, River Mardle. As agricultural topsoils are unlikely to be contaminated with metals, the likelihood of uncontaminated sediment diluting metal concentrations in riverbed sediments is reduced in situations where the catchment was dominated by rough grazing.

The delivery of sediment from sources other than mine workings/waste facilities to the river is enhanced in catchments with a high number of road crossings and/or road drains.

8.3 Retention of contaminated sediment at the site

The retention of contaminated sediment at a site is likely to increase the risk to ecology, with risk increasing where more contaminated sediment is retained. The extent to which a site retains fine sediment can be predicted from stream power (Naden et al., 2016). As most of the annual load of suspended sediment is carried during high flows, stream power should be calculated using the median annual flood (similar in return period to bankfull flow) which can be estimated from catchment characteristics (catchment area, average annual rainfall, flood attenuation due to reservoirs and lakes, and baseflow index), using the formula

$$\Omega = \rho g\, Q_{MED}\, S$$

where Ω is stream power (W/m), ρ is density of water (kg/m^3), g is acceleration due to gravity (m/s^2), Q_{MED} is median annual flood (m^3/s), S is channel slope (m/m). Of these parameters, channel slope is the easiest to determine, with lower channel slope associated with increased retention of sediment. Hence, we suggest the following influence,

10) *Slope of stream channel*

Nevertheless, as the results presented in Sections 4.3.4 and 4.3.5 indicate that the bioavailability of metals from sediment is related to concentration in the sediment rather than total load (concentration x mass of fine sediment), the influence of other sediment sources in the catchment is important. Where large amounts of sediment from other sources are retained, the effect of contaminated sediment from metal mine sources can be diluted. Hence, the footprint of the metal mine facility within the catchment is important. It should be noted that proximity to the mine facility is not a good predictor of the risk to ecology from contaminated sediment: with distance downstream from the mine facility metal concentrations and bioavailability tended to increase in some catchments (e.g. M, South Tyne), decrease in some (e.g. J, Afon Ystwyth), and appeared unrelated in others.

8.4 The influence of environmental conditions at the site on bioavailability of metals from contaminated sediment

From the results of Section 4.3.4d it is clear that pH has a significant influence on bioavailability of metals from the sediment, with reduced bioavailability at higher pH.

11) *pH of river water*

From the results of the controlled laboratory experiment (Section 7), it is apparent that the organic matter content of sediment increases the bioavailability of metals.

12) *Organic content of the sediment*

The results of the controlled laboratory experiment (Section 7) did not indicate a strong influence of water hardness and no effect of resuspension, although these may be important in the field.

The results of the controlled laboratory experiment (Section 7) demonstrated that contaminated riverbed sediment can act as a source of bioavailable metals in the absence of other sources, suggesting that remediation of sediment is likely to be required in addition to treatment of mine water discharge at sites where river bed sediments contain high concentrations of metals.

8.5 Summary of simple rules

We have attempted to identify a series of criteria that will help identify whether a site is likely to be at risk from metals associated with sediments. These criteria are summarised as a checklist of yes/no questions (Table 8.1), which could be used to identify situations where the risk to ecology from sediment from non-coal mines is enhanced. As the risk will vary at different points in the catchment downstream of mine facilities, the checklist should be used to determine the total risk score for a river site. We suggest that sites with a total risk score ≥10 are likely to be at high risk of impact from sediment contaminated by mine waste, sites with a total risk score of 5 – 9 are likely to be at moderate risk of impact from sediment contaminated by mine waste. Sites with scores below 5 are not likely to be at risk.

Table 8.1 Checklist to identify sites at high risk of sediment contaminated by mine waste.

		Marks	Risk Score
1	*The concentration of metals is high in the mine sediment sources/waste facilities material.*	3	
2	*Mine sediment sources/waste facilities comprise a high proportion of fine-grained material.*	1	
3	*Mine sediment sources/waste facilities are immediately adjacent to flowing watercourses.*	1	
4	*The area surrounding mine sediment sources/waste facilities has a high gradient.*	1	
5	*Mine sediment sources/waste facilities show indications of erosion.*	3	
6	*Mine sediment sources/waste facilities lack surface vegetation.*	3	
7	*Pollution mitigation measures including settling ponds are lacking or have failed.*	3	
8	*The mine sites have a large footprint within the catchment, i.e. the mine facility comprises a large proportion of the catchment (e.g. >2% catchment area).*	1	
9	*There is a low supply of sediment from other, uncontaminated sources, e.g. the river has few (≤10) road crossings/road drains, urban development is low (<5%) in the catchment, the catchment is dominated (> 50%) by rough grazing.*	1	
10	*The channel slope is low (< 15m km^{-1})*	1	
11	*pH of river water is low (pH < 6)*	3	
12	*The organic content of riverbed sediment is high*.	1	
	Total Risk Score		

9 Future research

Objective 7a
To provide guidance on the further evidence required to establish where mining-contaminated sediment gives rise to ecological impact or poses a significant risk.

Here we have reviewed available evidence and demonstrated experimentally that sediment contaminated with metals from abandoned mines presents a risk to ecology in the absence of other sources of metals. We have also used existing field data to determine the relationship between the extent of contamination and ecological damage, and thus identified conditions where sediments contaminated with metals exceed ecologically safe limits. Furthermore, we have developed a new invertebrate based biotic index that can be used to identify sites where mine impacts, particularly from contaminated sediment, represents a risk to ecology. However, as with all research some questions remain unanswered. Here we highlight areas where knowledge gaps are hindering our ability to predict the best mitigation strategy for rivers impacted by mine waters.

1) Further development and testing of the biotic index.

Although we have used existing data to test the MetTol index, the constraints on the data available have limited this assessment. In particular, we were not able to find a suitable data set that contained information on sediment metal concentrations. For a full independent test, we suggest that further data should be collected to specifically test, and if necessary, refine the index. These data would comprise information on sediment and water metal concentrations, biomonitor body burdens, and invertebrate community composition from sites that were not used in this study. As well as providing a full independent test of the index, such a dataset could be used to establish the index values (as Ecological Quality Ratios) that correspond to WFD classification boundaries and/or environmentally safe limits of sediment contamination. This work would provide further verification that the index can be used to identify sites where sediment is contaminated with metals from past mining activities.

2) Inclusion of biotic index into the River Invertebrate Classification Tool

For full operational use, the MetTol index should be incorporated into the River Invertebrate Classification Tool (RICT), the WFD tool used to assess ecological quality using invertebrates. Classification boundaries and uncertainty estimates would need to be derived (possibly through 1 above) to enable assessments. Once the MetTol index has been incorporated into RICT, assessments of the extent of ecological damage caused by metal contamination could be made during routine assessments of biology.

3) Field scale experiments

We suggest that manipulative experiments undertaken in field scale artificial channels would provide a further robust test of the impact of metal contaminated sediment on ecology (and of the MetTol index). Such experimental facilities could be set up streamside in situations where the water was not impacted by metal mining (e.g. upstream of failing mine facilities or downstream of mine water treatment facilities) but be filled with contaminated sediment (of varying composition). Such an approach would provide an assessment of the extent to which sediment is a source of bioavailable metals under field conditions and establish the conditions required to reduce this risk.

4) Establish impact of contaminated sediment on recovery.

In order to determine the extent to which contaminated sediment constrains recovery after treatment facilities for mine water discharges have been established, it would be useful to monitor any changes in bioavailability (i.e. change in biomonitor body burden), and concentrations of metals in sediment and water that occur over time downstream of treatment facilities post implementation. Thus, the extent to which the biota and sediment respond to treatment of mine water would be established. Similarly, it would be useful to determine the influence of contaminated sediment on recovery after the treatment of mine waste sediment sources (e.g. by capping of spoil heaps).

5) Further controlled laboratory experiments.

In order to establish the conditions that influence the bioavailability of metals from sediment and the mechanisms of uptake more precisely, it would be useful to undertake further controlled laboratory experiments. For example, to establish more precisely the influence of pH (and alkalinity) on metal bioavailability from the sediment, to establish differences in bioavailabilities among metals, and the use organic matter spiked with metals to determine more precisely the route of uptake. It would also be useful to undertake experiments to assess the relative importance of uptake of metals from water and sediments (e.g. using clean sediments and contaminated water), and to establish if sediment plays a role in the uptake of dissolved metals from the water.

6) Bioaccumulation

Here we have investigated the relationships between sediment contaminated with metals from abandoned metal mines and invertebrates. Impacts at higher trophic levels (i.e. fish) were only explored using existing data. As aquatic invertebrates comprise a large proportion of the diet of fish in rivers impacted by abandoned metal mines, the role that invertebrates play in the uptake of metals by fish would be of key interest. It is typically assumed that fish acquire metals from the environment in dissolved form via the gills. Uptake of metals via the gut from food particles (i.e. aquatic invertebrates), which in turn acquire metals from the sediment, presents an alternative uptake mechanism and one that is likely to be of increasing importance as mine waters are treated. As salmonids typically occur in the rivers draining geologies associated with metal mining, the concentration of metals in fish tissues has potential implications for human health should these fish enter the human food chain. Thus, the extent to which fish tissues are contaminated with metals, and the route of uptake of these metals are of particular interest. A study of the food webs of metal mine impacted rivers, focussing on the bioaccumulation of metals from the sediment, would provide considerable understanding of the pathways of metal uptake into biota at impacted sites and, hence, the likely continued influence of metal contaminated sediment on fish after mine waters have been treated.

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Appendix 1 EA/NRW water chemistry determinands

Det_co	Determinand				
0061	pH - AS pH UNITS		0209	POTASSIUM DISSOLVED - as K	(mg/l
0076	TEMPERATURE WATER	CELSIUS	0207	SODIUM - as Na	(mg/l
0062	CONDUCTIVITY @20C	µS/cm	0205	SODIUM DISSOLVED - as Na	(mg/l
0077	CONDUCTIVITY @25C	µS/cm		PCP	(µg/l)
0163	HARDNESS CALCIUM	(mg/l)	6056	PCB	(mg/l
0164	HARDNESS MAGNESIUM	(mg/l)	9050	1,2,3-Trichlorobenzene	(µg/l)
0158	HARDNESS TOTAL - as	(mg/l)	9051	1,2,4-Trichlorobenzene	(µg/l)
0162	ALKALINITY pH 4.5 - as	(mg/l)	9052	1,3,5-Trichlorobenzene	(µg/l)
0081	OXYGEN DISSOLVED %	%		1,1,1-Trichloroethane	(µg/l)
0082	OXYGEN DISSOLVED - as O	(mg/l)		1,2-Dichloroethylene	(µg/l)
9901	OXYGEN DISSOLVED	%		2,4-Ethenoic	(µg/l)
9924	OXYGEN DISSOLVED	(mg/l)		2-Chlorophenol	(µg/l)
0085	BOD ATU as O_2	(mg/l)	6404	TETRACHLOROMETHANE	(mg/l
0111	AMMONIA - as N	(mg/l)		Aldrin	(µg/l)
0119	AMMONIA UN-IONISED	(mg/l)		Atrazine	(µg/l)
0068	TURBIDITY	FTU		Azinphos	(µg/l)
0135	SOLIDS SUSPENDED	(mg/l)		Azinphos-ethyl	(µg/l)
0143	SOLIDS NON-VOLATILE	(mg/l)		Chlorfenvinphos	(µg/l)
0116	NITROGEN TOTAL	(mg/l)		DDD pp	(µg/l)
3683	NITROGEN TOTAL	(mg/l)		DDE pp	(µg/l)
0117	NITRATE - as N	(mg/l)		DDE pp'	(µg/l)
0118	NITRITE - as N	(mg/l)		DDT op	(µg/l)
9194	NITROGEN TOTAL	(µg/l)		DDT op'	(µg/l)
9195	PHOSPHORUS TOTAL	(µg/l)		DDT pp	(µg/l)
0348	PHOSPHORUS TOTAL - as P	(mg/l)		DDT pp'	(µg/l)
0180	ORTHOPHOSPHATE - as P	(mg/l)		Diazinon	(µg/l)
0191	ORTHO PHOSPHATE	(mg/l)		Dichlorvos	(µg/l)
7692	PHOSPHORUS SOLUBLE	(mg/l)		Dieldrin	(µg/l)
0192	PHOSPHATE	(mg/l)		Dimethoate	(µg/l)
0947	CHLOROPHYLL (A + B)	(µg/l)		Endosulfan A	(µg/l)
0172	CHLORIDE ION - as Cl	(mg/l)		Endosulfan Total	(µg/l)
0050	LEAD - as Pb	(µg/l)		Endrin	(µg/l)
0052	LEAD DISSOLVED - as Pb	(µg/l)		Fenitrothion	(µg/l)
0108	CADMIUM - as Cd	(µg/l)		Fenthion	(µg/l)
0106	CADMIUM DISSOLVED - as	(µg/l)		HCB	(µg/l)
3164	CHROMIUM - as Cr	(µg/l)		HCBD	(µg/l)
3409	CHROMIUM DISSOLVED - as	(µg/l)		HCH	(µg/l)
6455	ZINC - as Zn	(µg/l)		Isodrin	(µg/l)
3408	ZINC DISSOLVED - as Zn	(µg/l)		Linuron	(µg/l)
6462	NICKEL - as Ni	(µg/l)		Malathion	(µg/l)
3410	NICKEL DISSOLVED - as Ni	(µg/l)		MBAS	(µg/l)
6452	COPPER - as Cu	(µg/l)		Mecoprop	(µg/l)
6450	COPPER DISSOLVED - as	(µg/l)		Mevinphos	(µg/l)
0105	MERCURY - as Hg	(µg/l)		Parathion	(µg/l)
0103	MERCURY DISSOLVED - as	(µg/l)		Parathion-methyl	(µg/l)
0287	ALUMINIUM - as Al	(mg/l)		Propetamphos	(µg/l)
0285	ALUMINIUM DISSOLVED - as	(mg/l)		Simazine	(µg/l)
0352	VANADIUM - as V	(mg/l)		TDE pp'	(µg/l)
0350	VANADIUM DISSOLVED - as	(mg/l)		Triazophos	(µg/l)
0356	ARSENIC - as As	(mg/l)		Trifluralin	(µg/l)
0354	ARSENIC DISSOLVED - as	(mg/l)			
0421	IRON - as Fe	(mg/l)			
0419	IRON DISSOLVED - as Fe	(mg/l)			
0403	MANGANESE - as Mn	(mg/l)			
0237	MAGNESIUM - as Mg	(mg/l)			
0235	MAGNESIUM DISSOLVED -	(mg/l)			
0211	POTASSIUM - as K	(mg/l)			

Appendix 2 Details of quantile regression

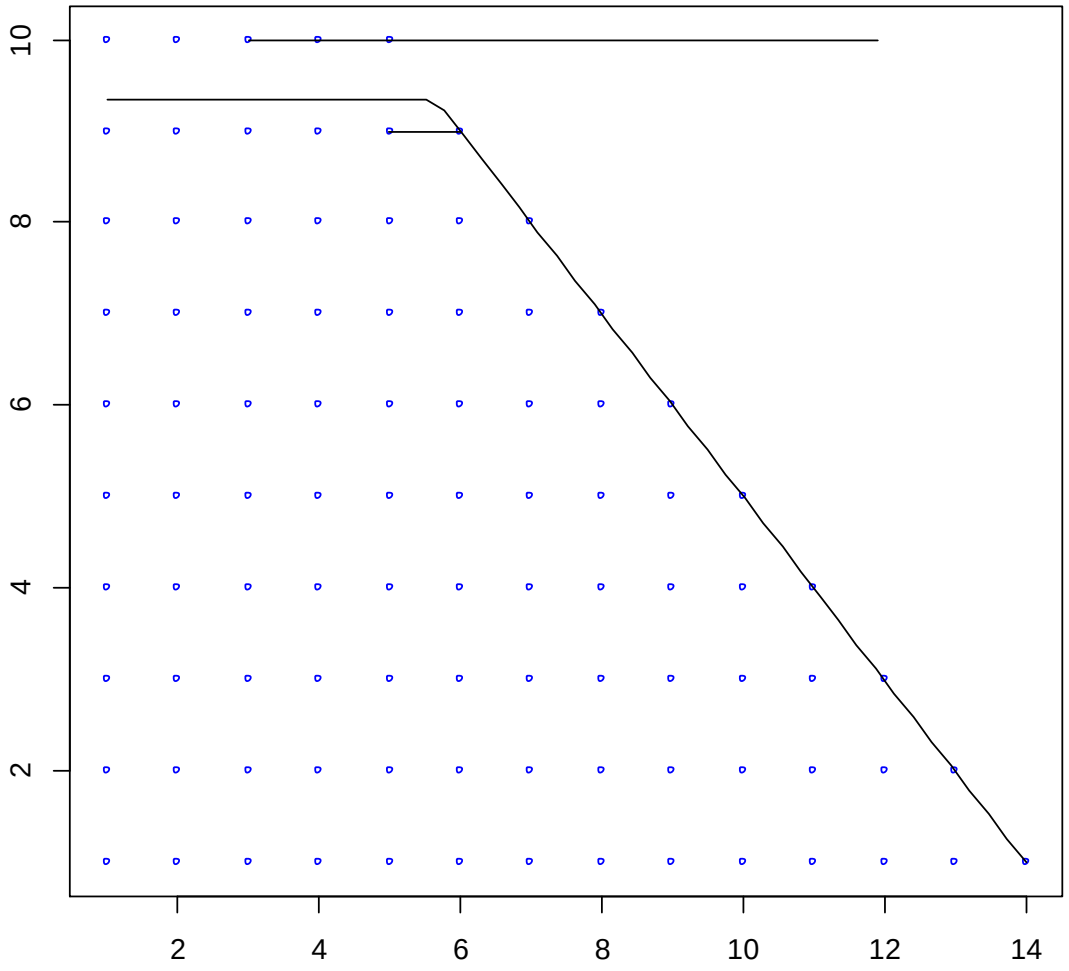
A2.1 Details of R script for threshold model

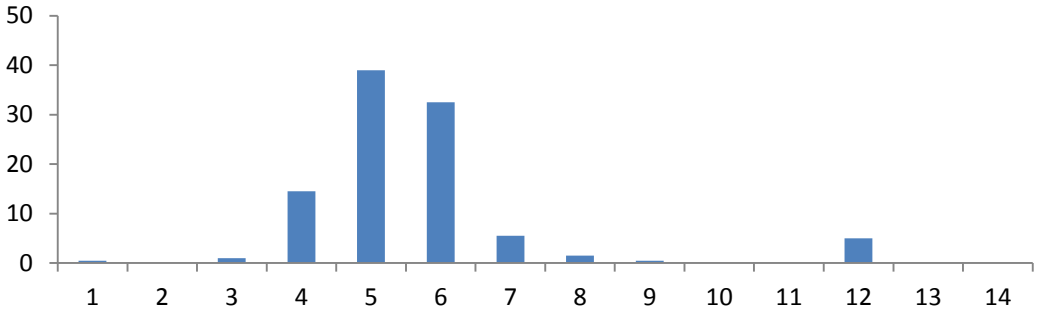
```
yy <- NTAXA
xx <- LCd
n <- length(xx) # sample size
resd1 <- rep(9999, n)
nlrq.f <- function(para){
        pre.yy <- para[1] +(para[2] * (xx - para[3]))*(xx > para[3])
        for (i in 1:n){
        resd1[i]<- ifelse(yy[i] >= pre.yy[i],
        tau1*(yy[i]-pre.yy[i]),(1-tau1)*(pre.yy[i]-yy[i]))
        }
        sum (resd1)
        }

library (ecolMod)  # required to use the function “pricefit”
# More details on this function are available in Soetaert and Herman (2009).

## Initial settings
# The quantile to be estimated
tau1 <- 0.95
ini.para <- c(20, -1, 1)

# The number of iterations
# Value determined from the results of trials

n.ite <- 50000

# Assign minimum and maximum values for the estimation of parameters
# Values assigned from the scatter plot and results of trials.
# Particularly for the threshold concentration, the range is set to be sufficiently large.
# Here is the example for Cd

min.para <- c(10, -100, -0.5)
max.para <- c(40, 100, 2.12)

# (max. and min. values for Cd are given, be aware that the range of y and slope need to be
appropriate too)
# (if par estimates return on the boundary, expand the range)

## Run the estimation

p1 <- pricefit(par=ini.para, func=nlrq.f, numiter=n.ite, minpar=min.para, maxpar=max.para )

p1$par
```

```
# The sum of weighted absolute deviation

p1$cost

p1$cl

## Compare the AIC values

# Threshold model
2*n*log(p1$cost/n)+2*3

# Exponential model
library(quantreg)
2*n*log(rq(yy~exp(xx),tau=0.95)$rho/n)+2*2

# Linear model
2*n*log(rq(yy~xx,tau=0.95)$rho/n)+2*2

# Null model
2*n*log(rq(yy~1,tau=0.95)$rho/n)+2*1
```

A2.2 Test data used to Illustrate Confidence Intervals

An artificial test dataset was constructed with one data point in each position 1-10, with the maximum declining by one with each step above 5. A curve was fitted by 95 %tile regression using the threshold model as detailed in section A2.1. The upper horizontal line 95% corresponds to the confidence intervals of the cut point established by bootstrap and the lower horizontal line to the 95% confidence intervals established by jackknife. The distribution of the bootstrap results are shown in the panel below.

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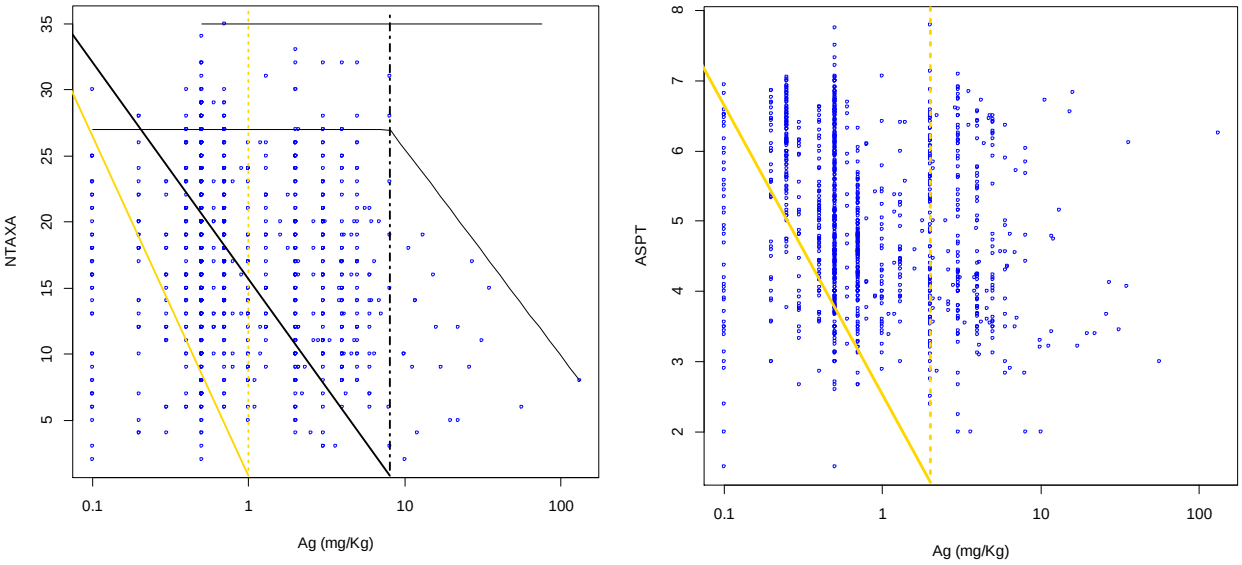
A2.3 Results of Quantile Model fits

i) Invertebrates

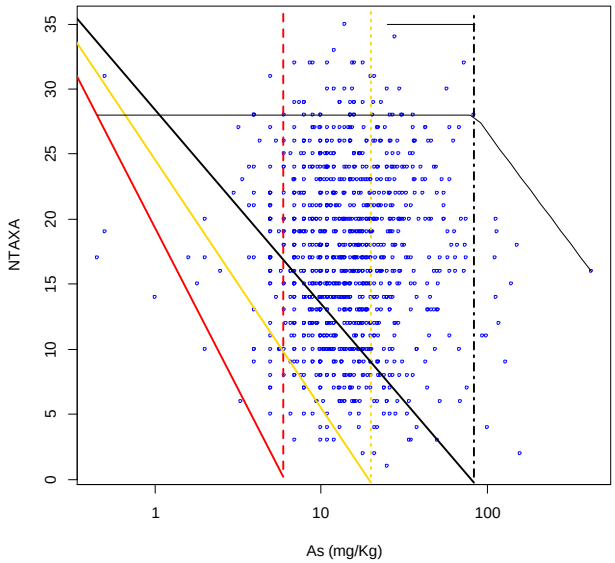
NTAXA and ASPT

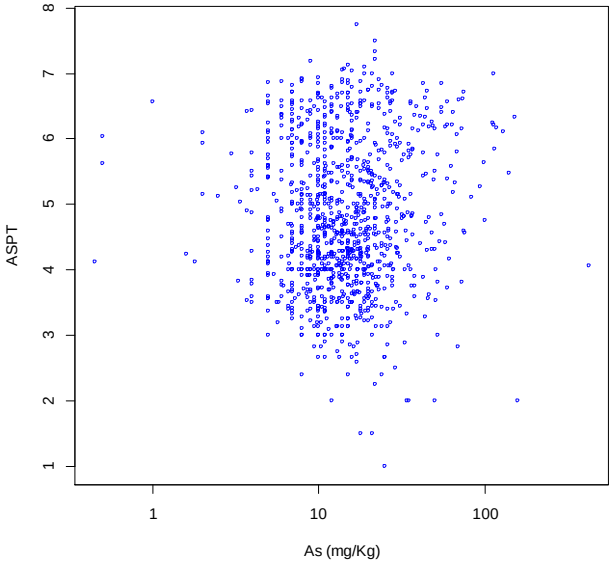
Fitted models shown: modelled threshold values indicated by dot-dashed black lines. Canadian interim sediment quality guidelines (CCME, 1999) shown with dashed red lines, ANZECC and ARMCANZ (2000) low trigger values shown with dotted yellow lines.

Silver

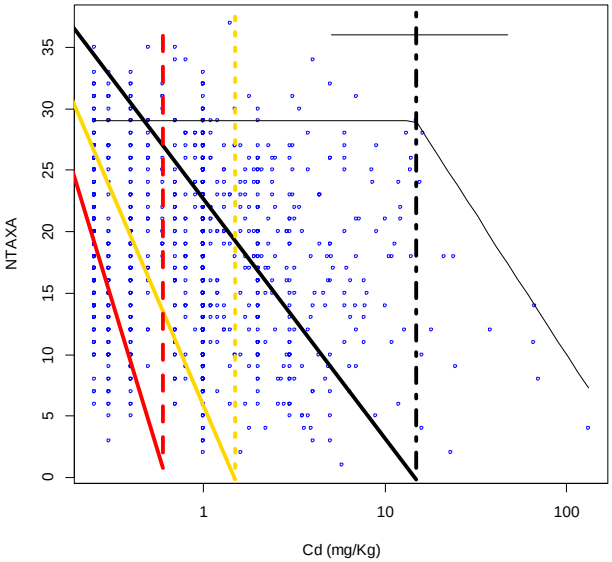


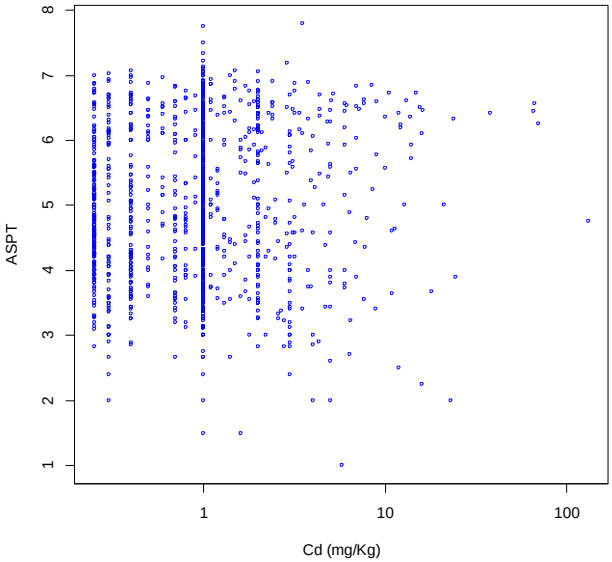
Arsenic



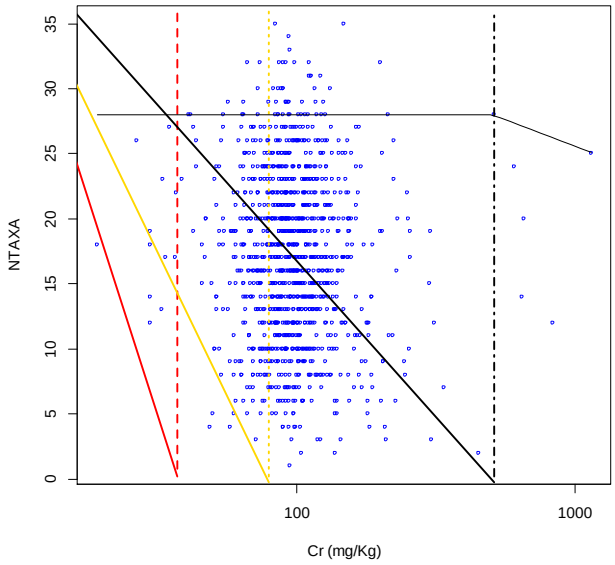


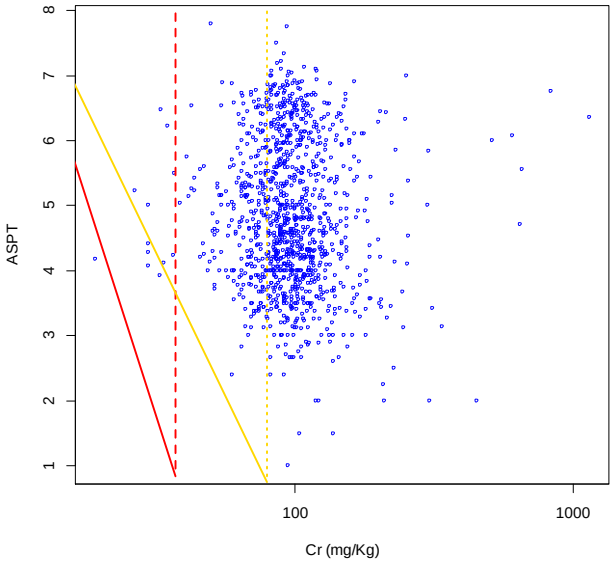
Cadmium



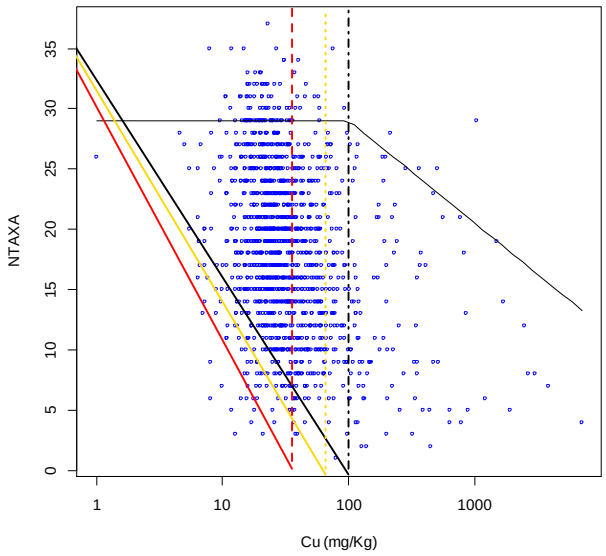


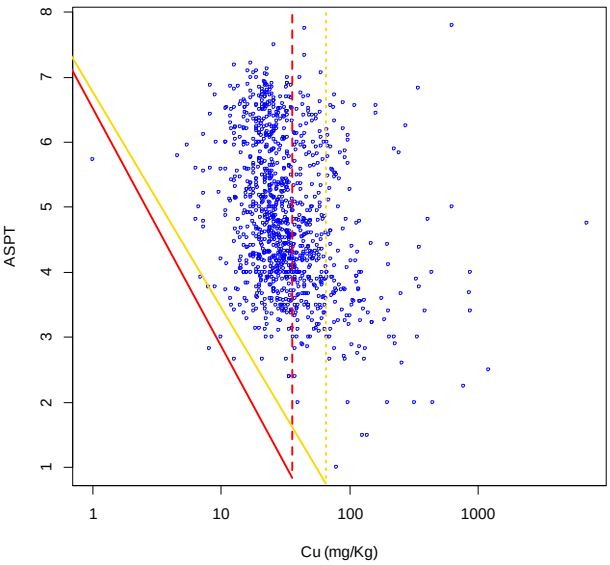
Chromium



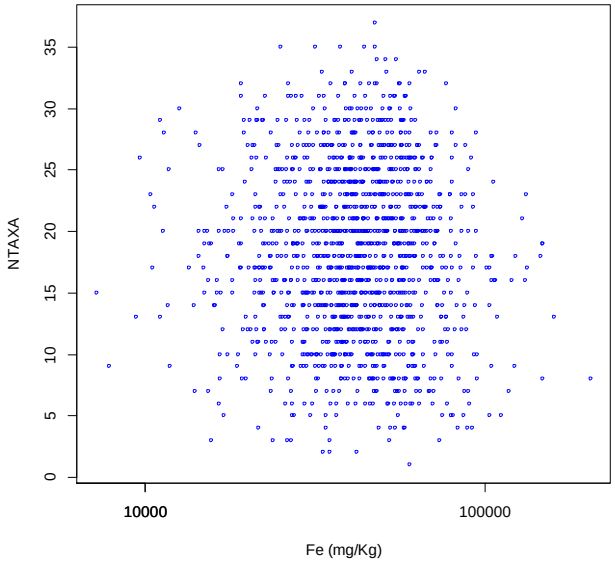


Copper





Iron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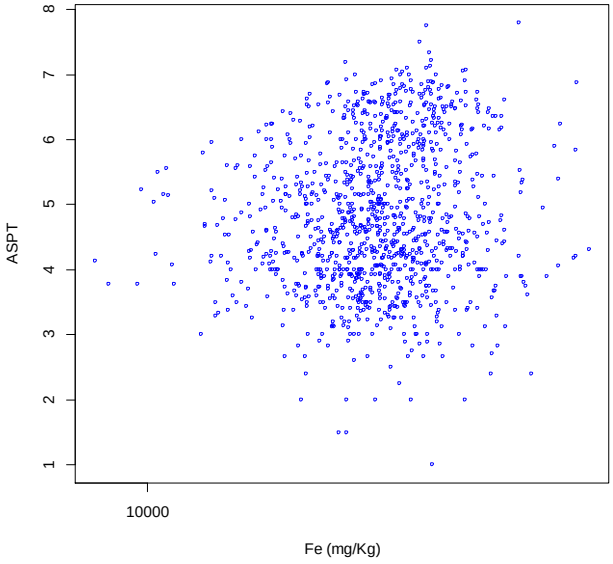
Fe (mg/Kg)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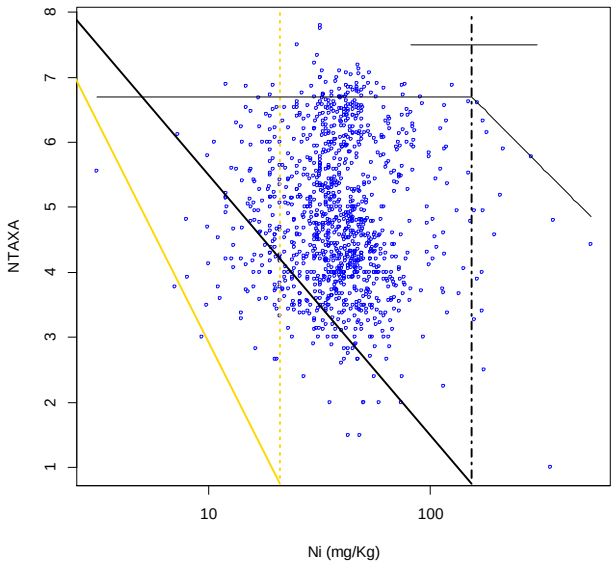
Fe (mg/Kg)

Nickel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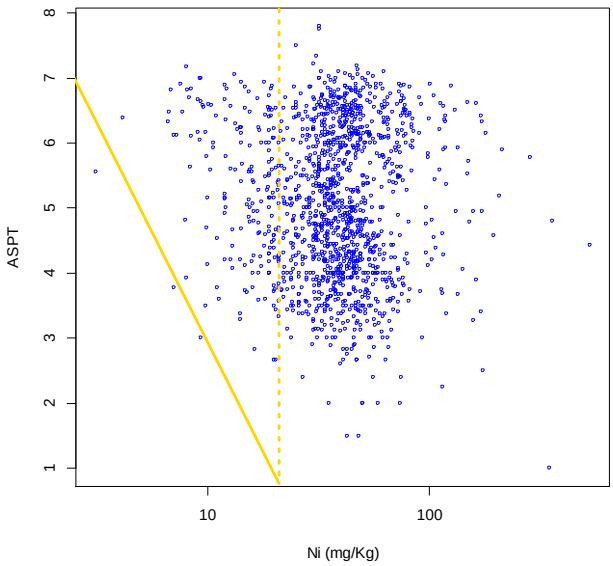
Ni (mg/Kg)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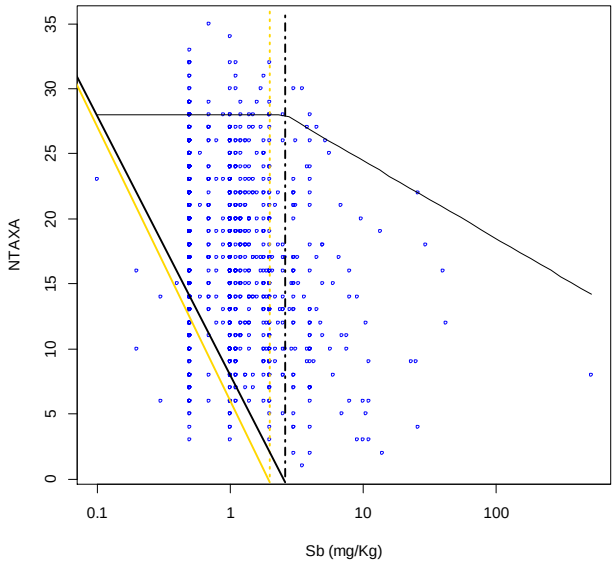
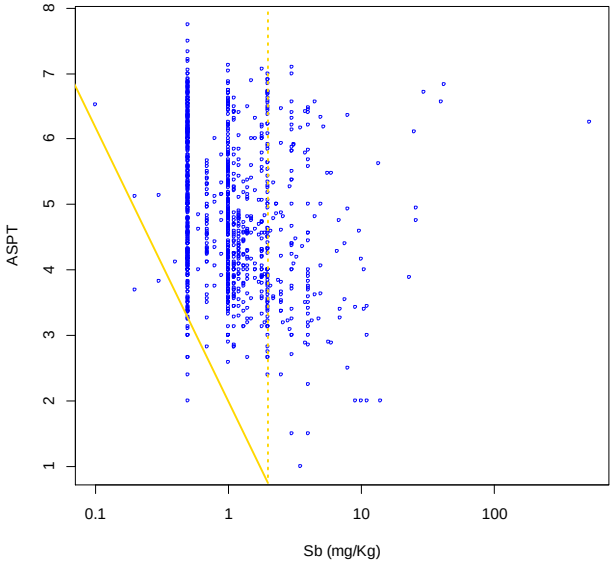
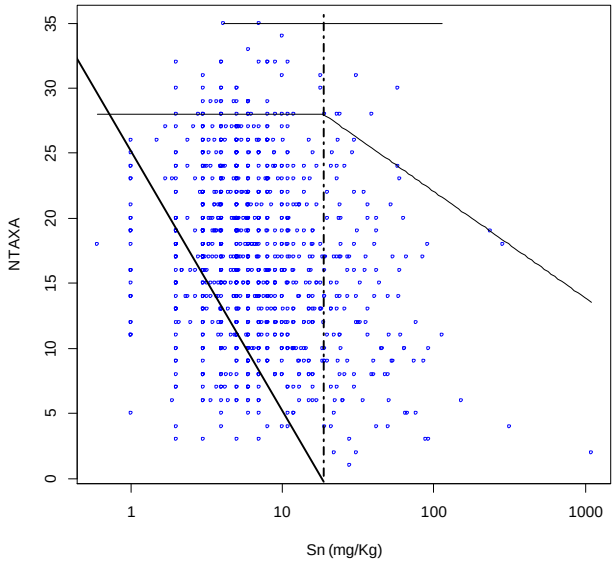
Ni (mg/Kg)

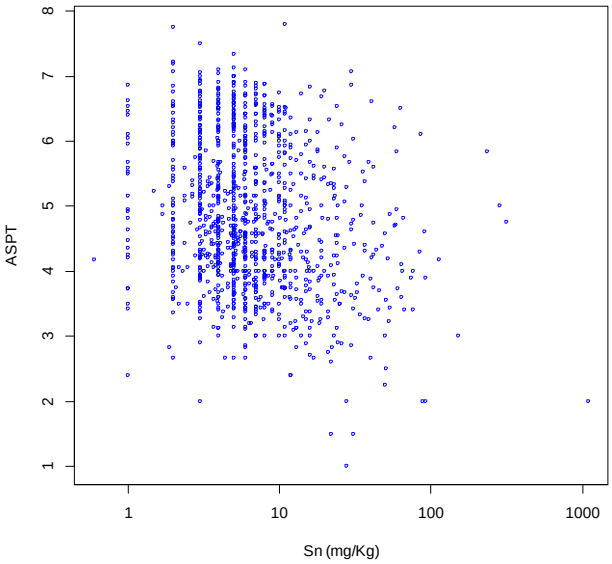
Antimony



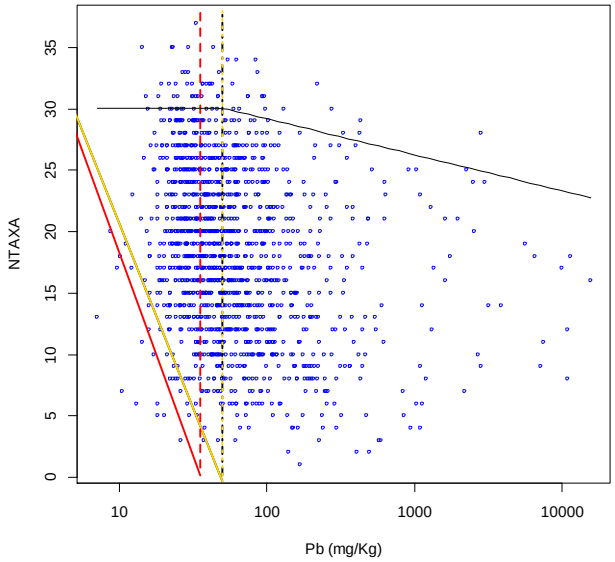


Tin





Lead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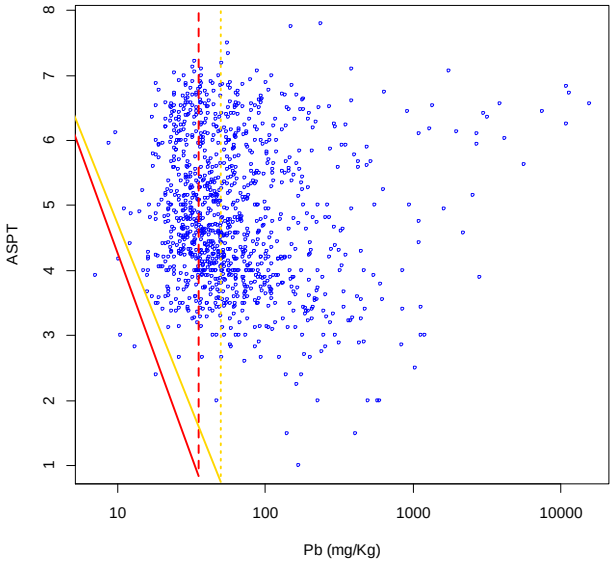
Pb (mg/Kg)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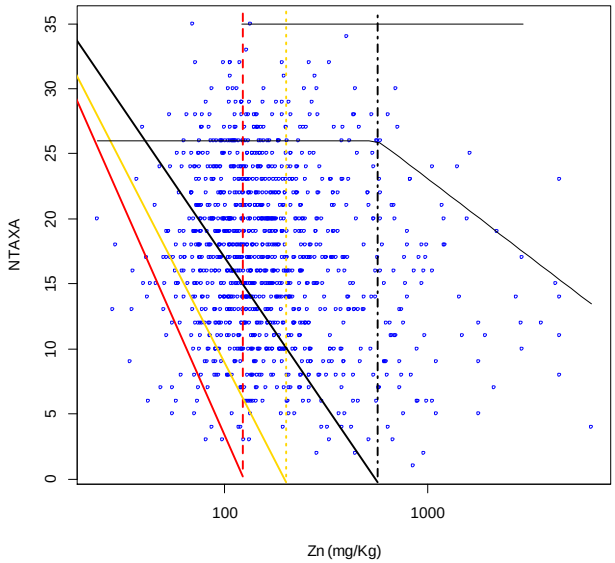
Pb (mg/Kg)

Zinc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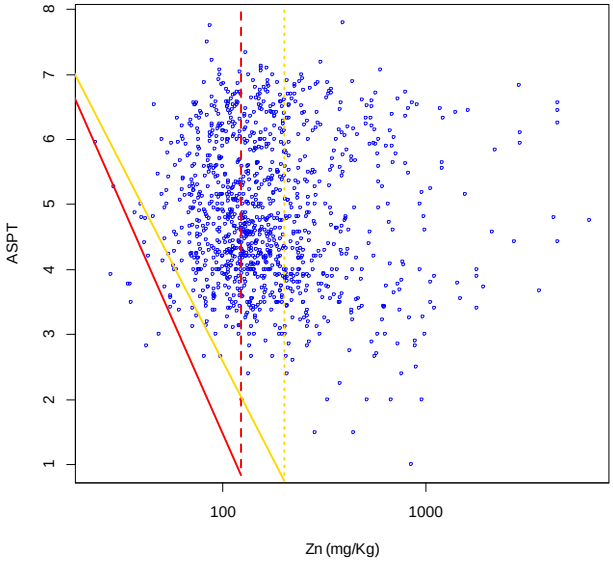
Zn (mg/Kg)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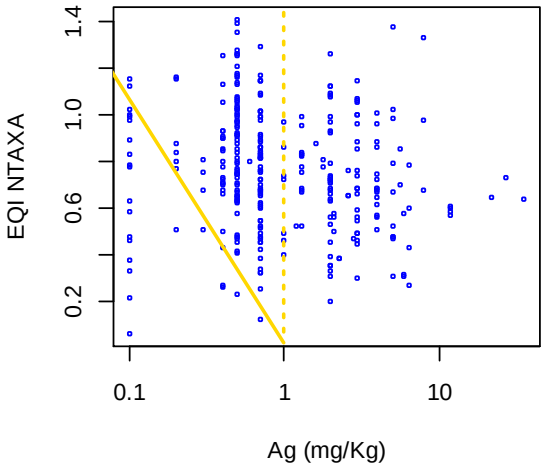
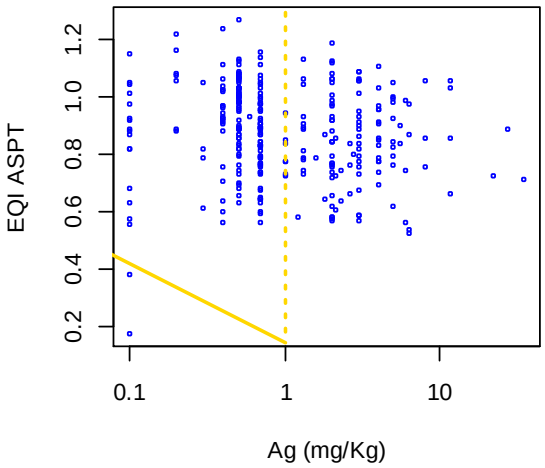
Zn (mg/Kg)

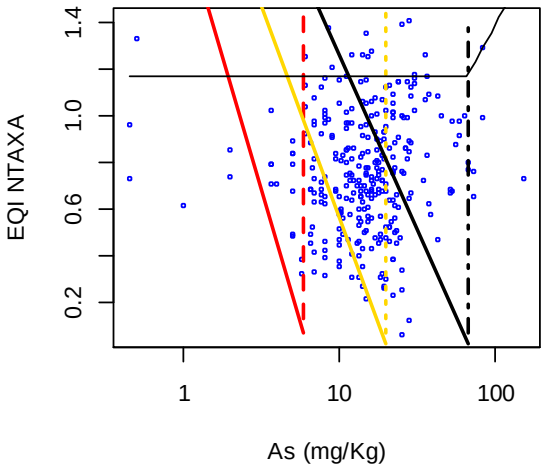
EQR NTAXA and EQR ASPT

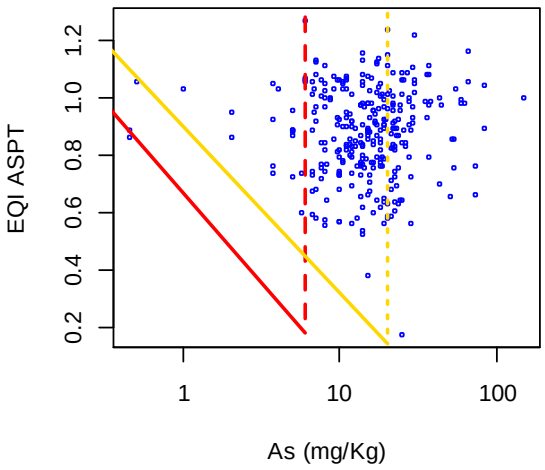
Silver



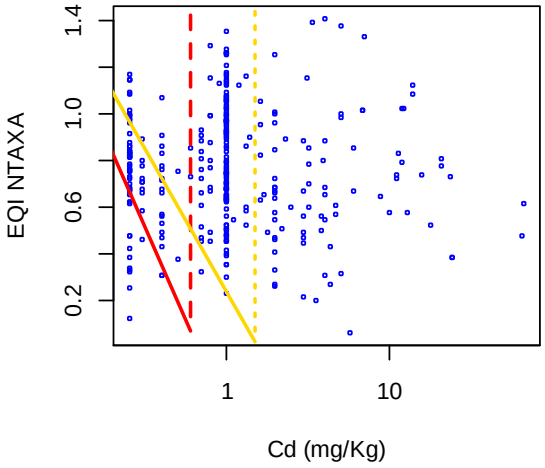


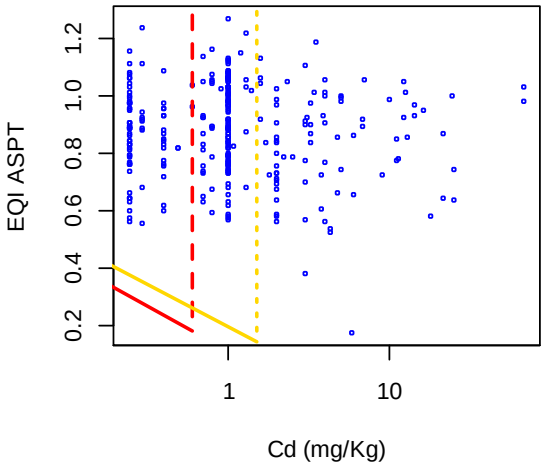
Arsenic



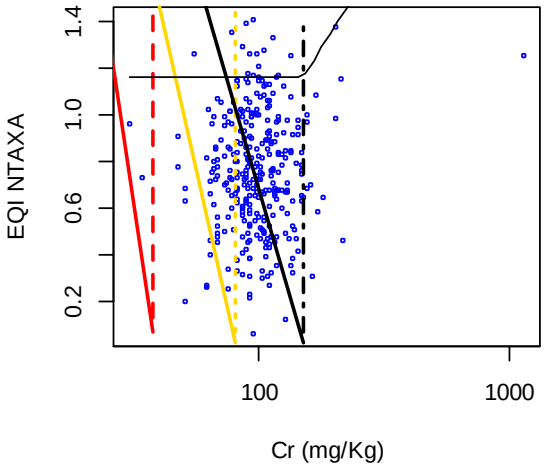


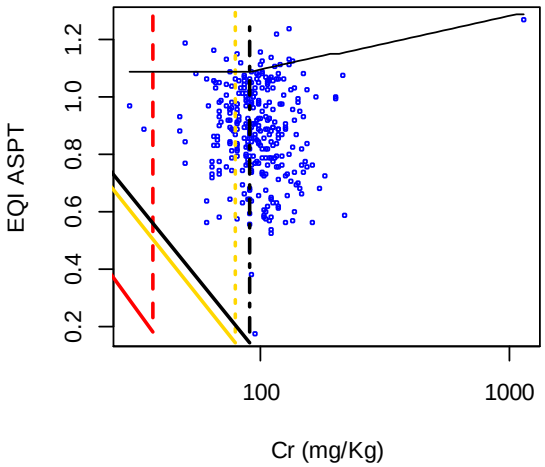
Cadmium



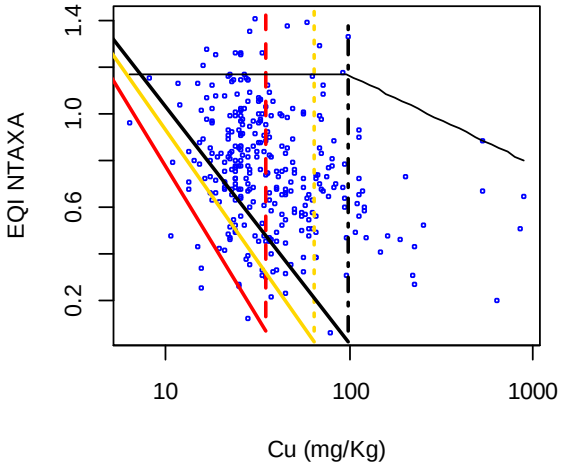


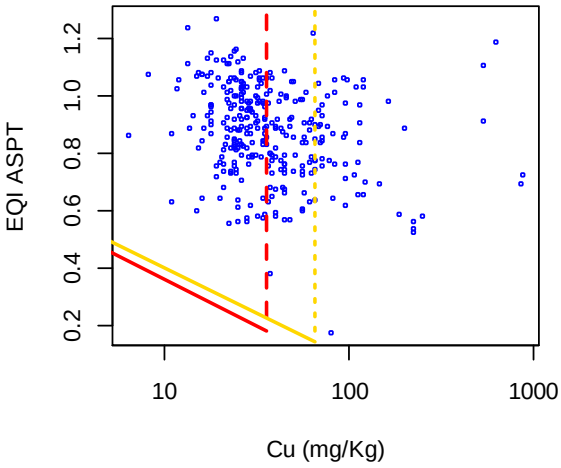
Chromium



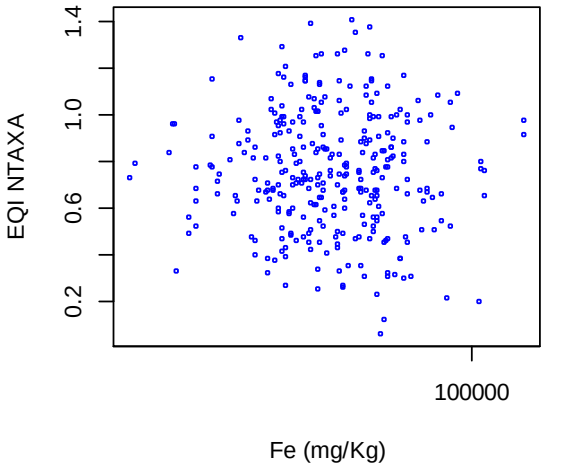


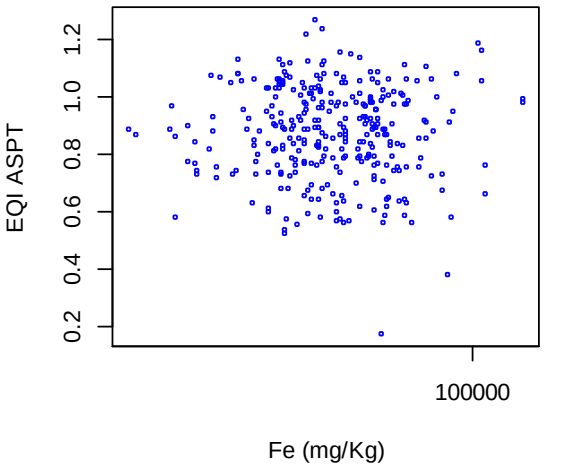
Copper


EQI NTAXA
0.2
0.6
1.0
1.4
10
100
1000
Cu (mg/Kg)

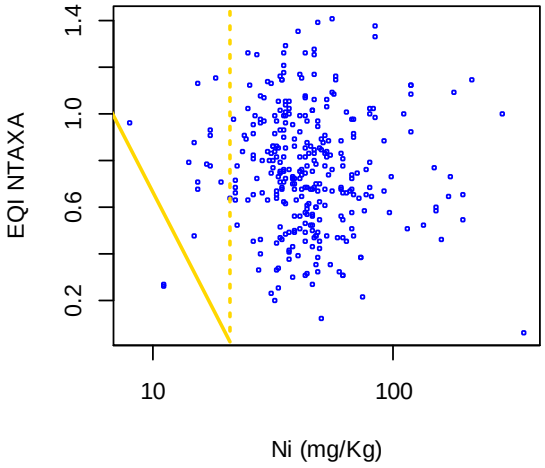

EQI ASPT
0.2
0.4
0.6
0.8
1.0
1.2
10
100
1000
Cu (mg/Kg)

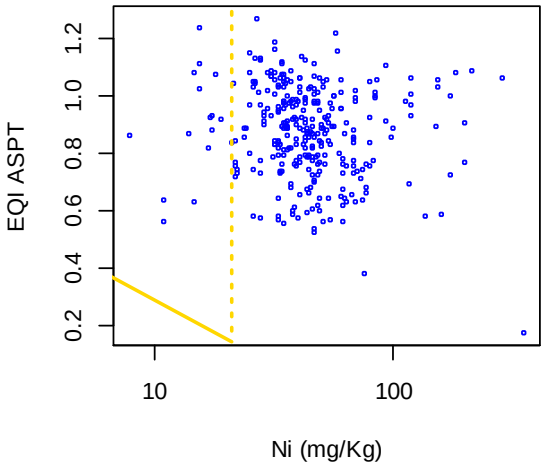
Iron


EQI NTAXA
0.2
0.6
1.0
1.4
100000
Fe (mg/Kg)

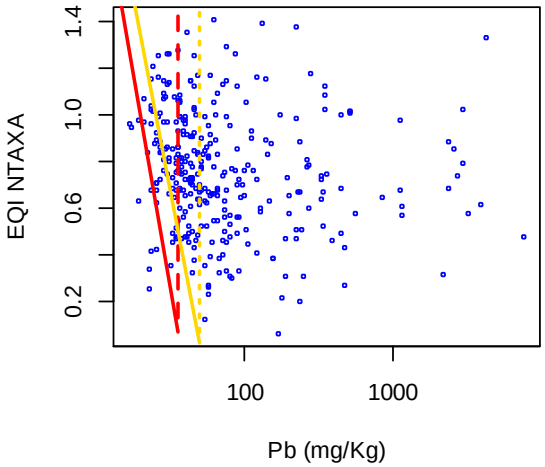

EQI ASPT
0.2
0.4
0.6
0.8
1.0
1.2
100000
Fe (mg/Kg)

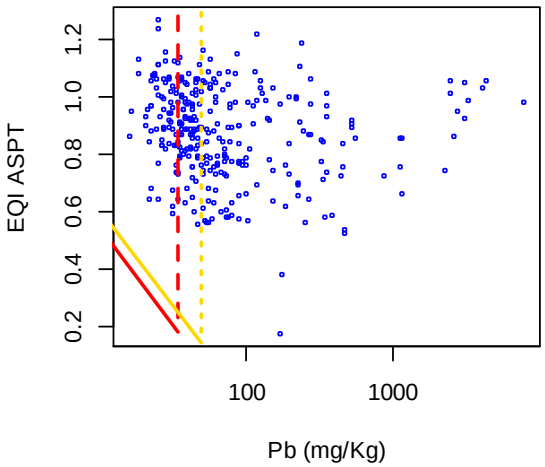
Nickel


EQI NTAXA
0.2
0.6
1.0
1.4
10
100
Ni (mg/Kg)

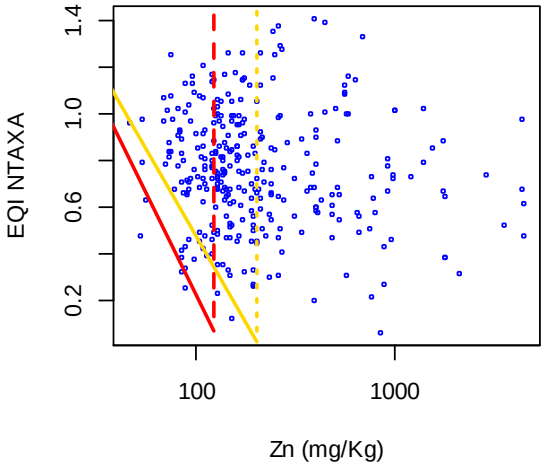

EQI ASPT
0.2
0.4
0.6
0.8
1.0
1.2
10
100
Ni (mg/Kg)

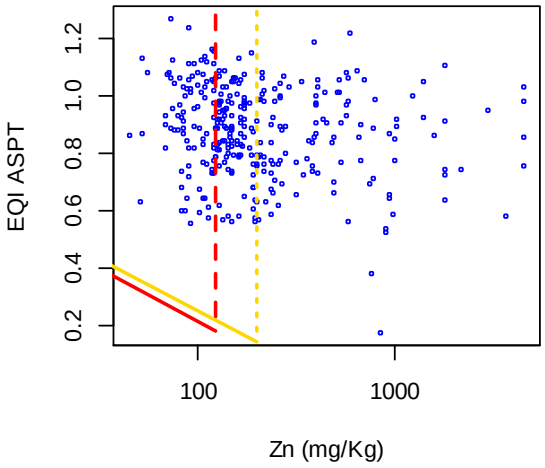
Lead


EQI NTAXA
0.2
0.6
1.0
1.4
100
1000
Pb (mg/Kg)


EQI ASPT
0.2
0.4
0.6
0.8
1.0
1.2
100
1000
Pb (mg/Kg)

Zinc

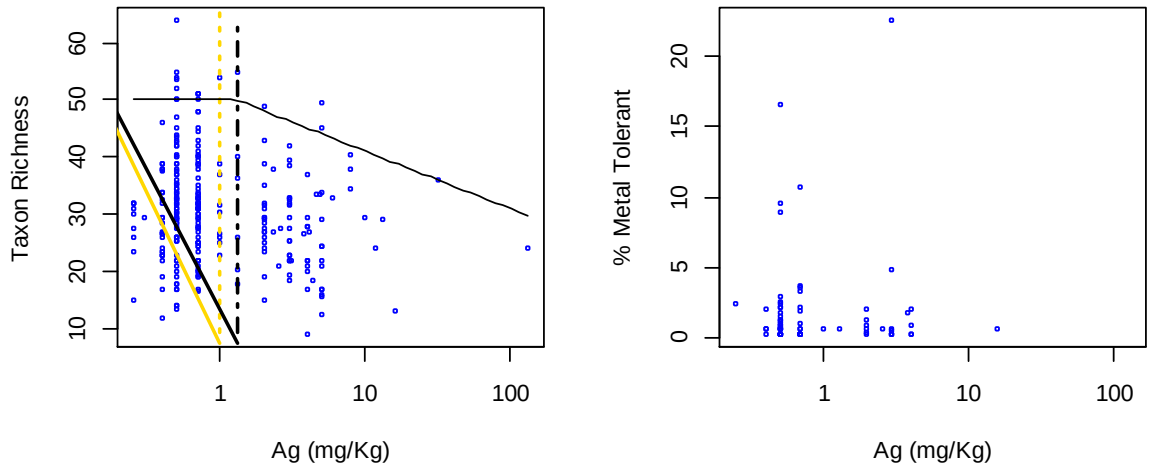

EQI NTAXA
0.2
0.6
1.0
1.4
100
1000
Zn (mg/Kg)

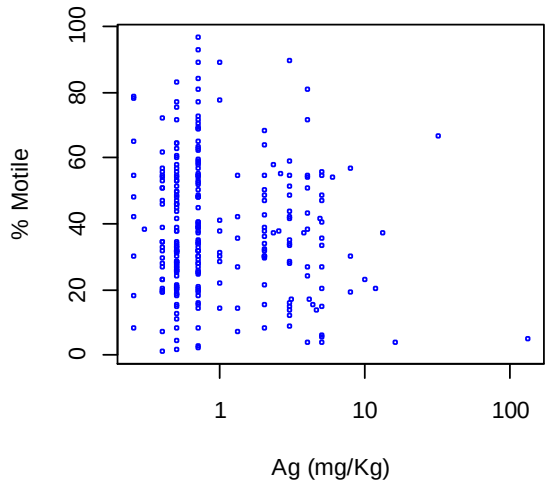

EQI ASPT
0.2
0.4
0.6
0.8
1.0
1.2
100
1000
Zn (mg/Kg)

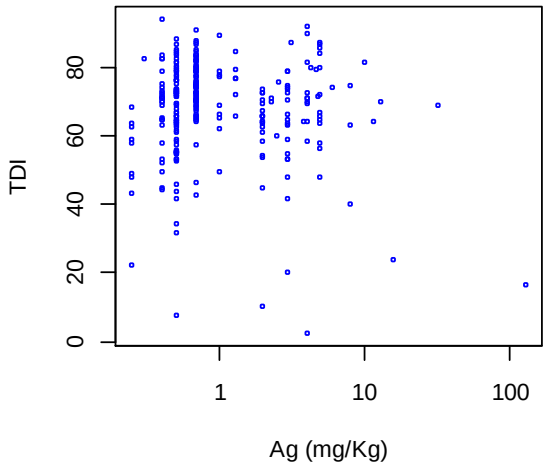
ii) Diatoms

Taxon Richness, % Metal tolerant taxa, % Motile taxa, and TDI

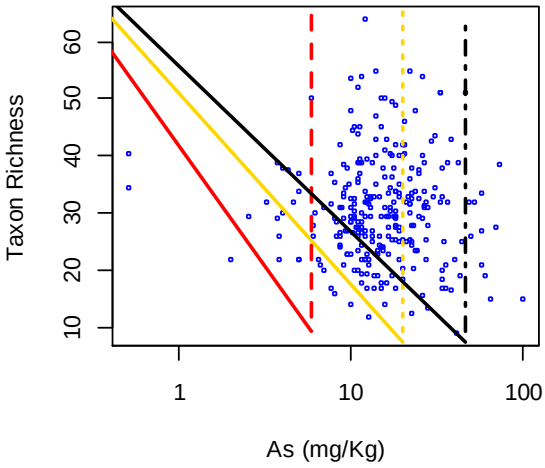
Silver

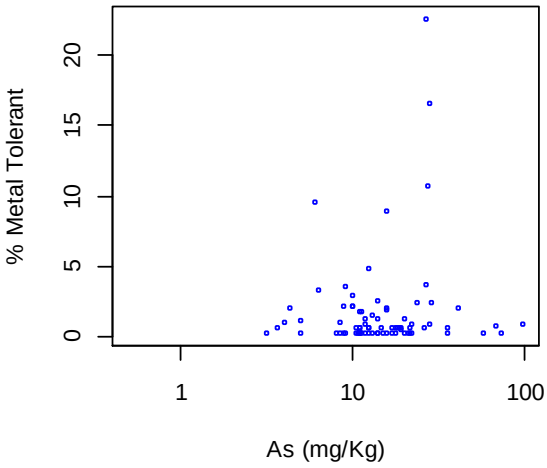


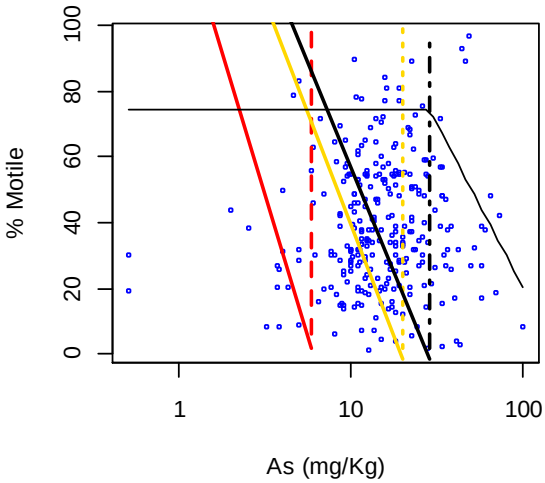


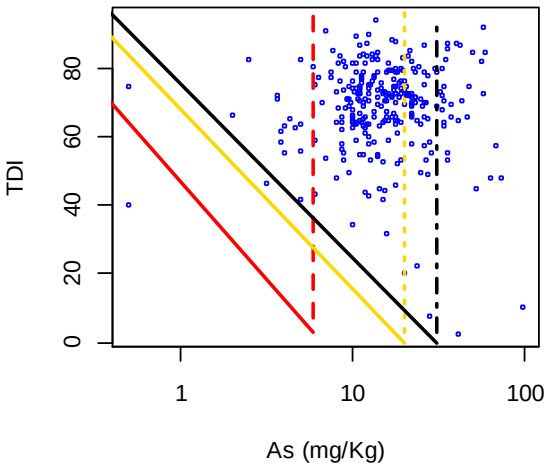


Arsenic

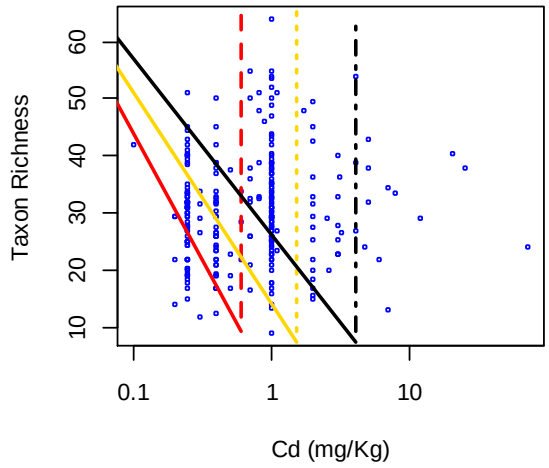

Taxon Richness
10
20
30
40
50
60
1
10
100
As (mg/Kg)

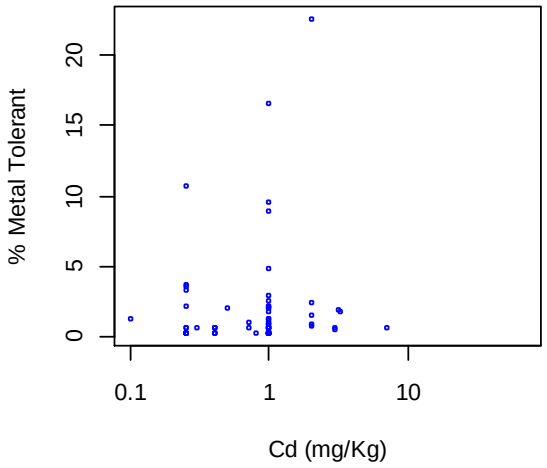

% Metal Tolerant
0
5
10
15
20
1
10
100
As (mg/Kg)

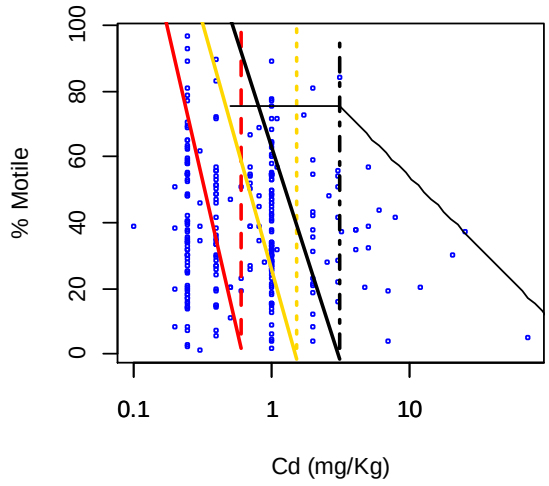

% Motile
0
20
40
60
80
100
1
10
100
As (mg/Kg)

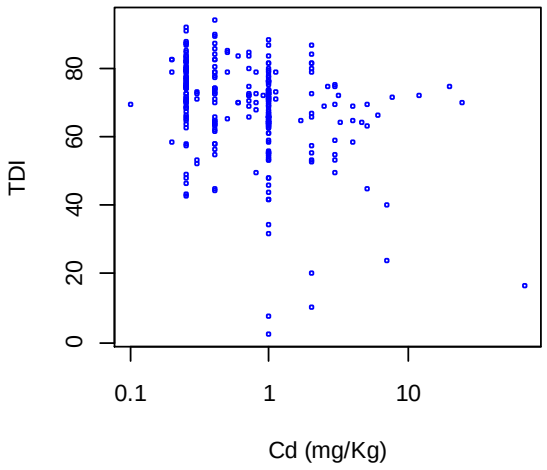

TDI
0
20
40
60
80
1
10
100
As (mg/Kg)

Cadmium

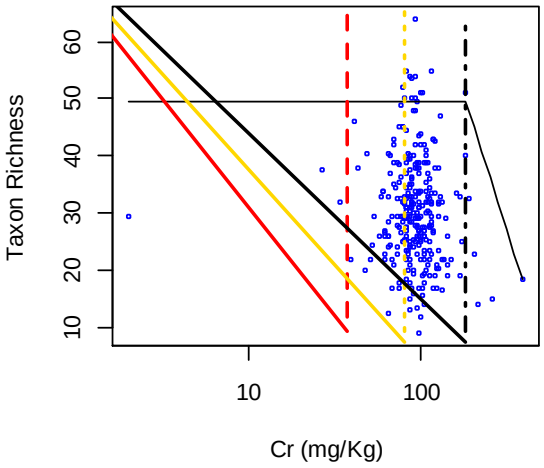

Taxon Richness
10
20
30
40
50
60
0.1
1
10
Cd (mg/Kg)

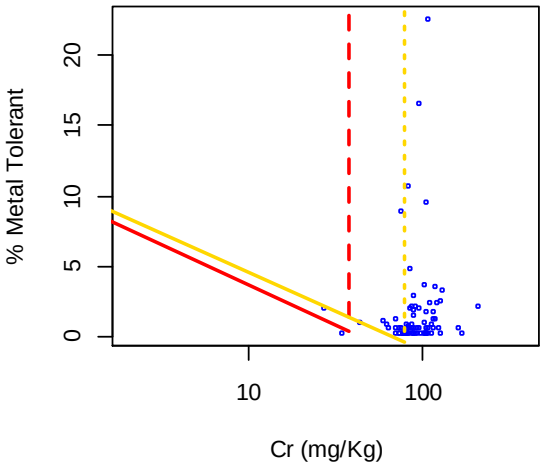

% Metal Tolerant
0
5
10
15
20
0.1
1
10
Cd (mg/Kg)

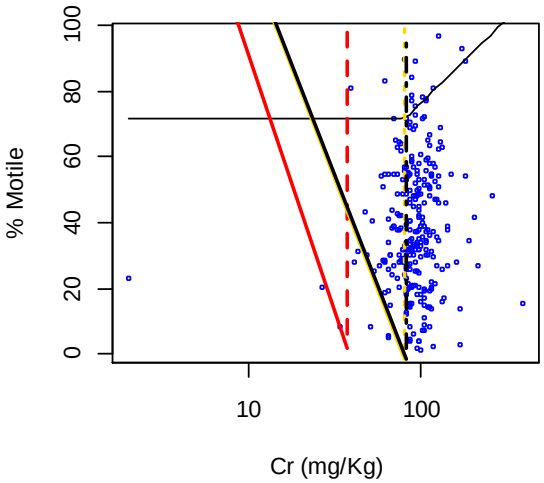

% Motile
0
20
40
60
80
100
0.1
1
10
Cd (mg/Kg)

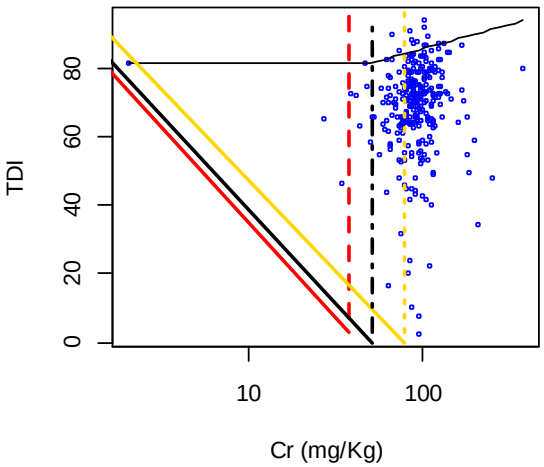

TDI
0
20
40
60
80
0.1
1
10
Cd (mg/Kg)

Chromium

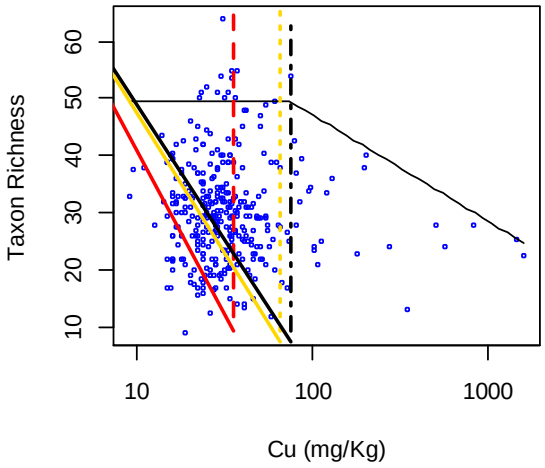

Taxon Richness
10
20
30
40
50
60
10
100
Cr (mg/Kg)

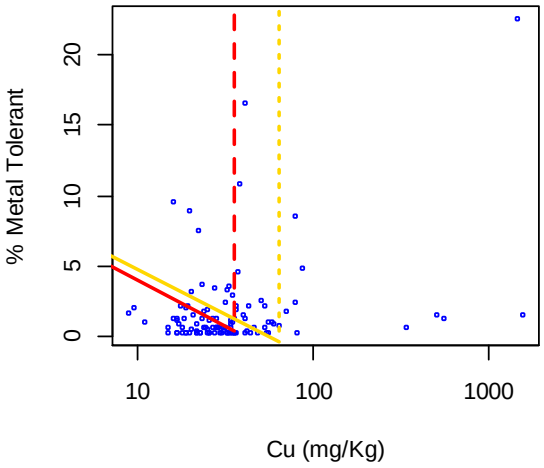

% Metal Tolerant
0
5
10
15
20
10
100
Cr (mg/Kg)

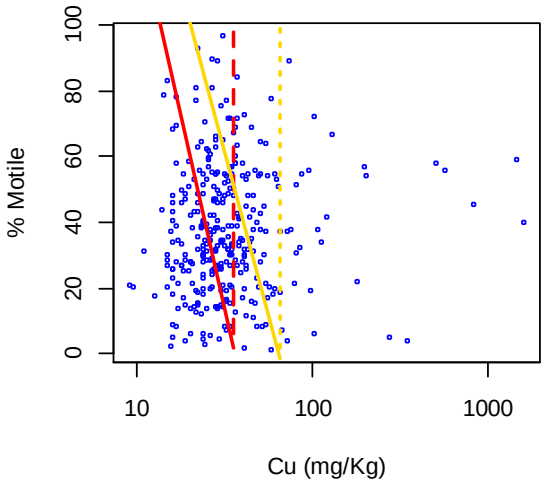

% Motile
0
20
40
60
80
100
10
100
Cr (mg/Kg)

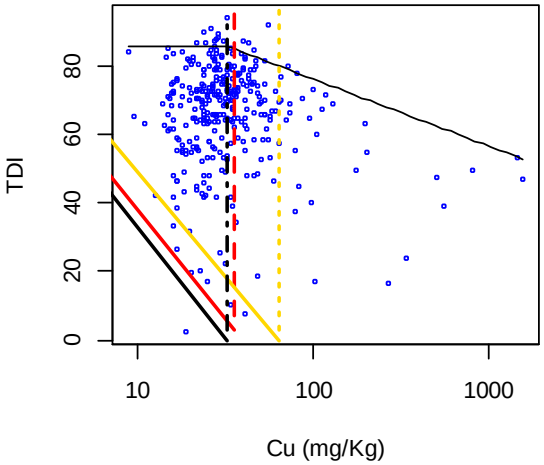

TDI
0
20
40
60
80
10
100
Cr (mg/Kg)

Copper

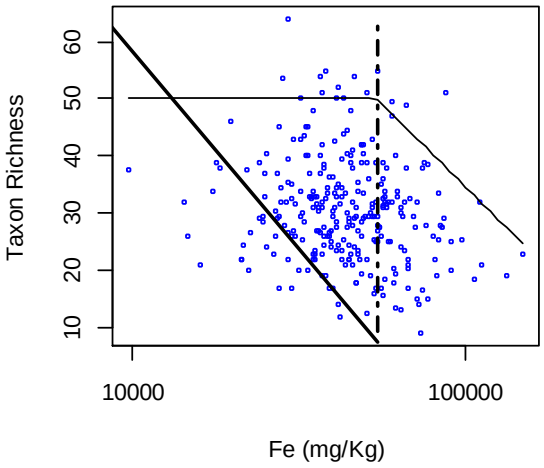

Taxon Richness
10
20
30
40
50
60
10
100
1000
Cu (mg/Kg)

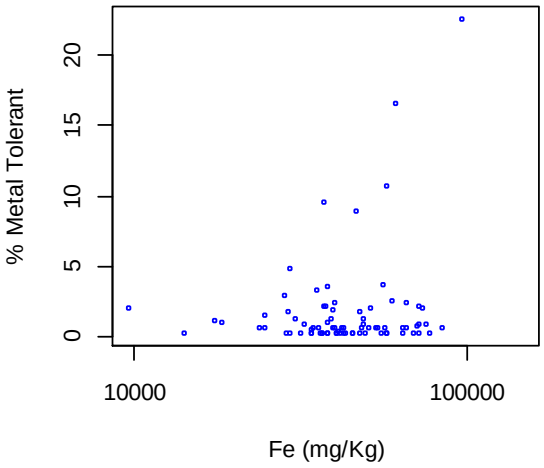

% Metal Tolerant
0
5
10
15
20
10
100
1000
Cu (mg/Kg)

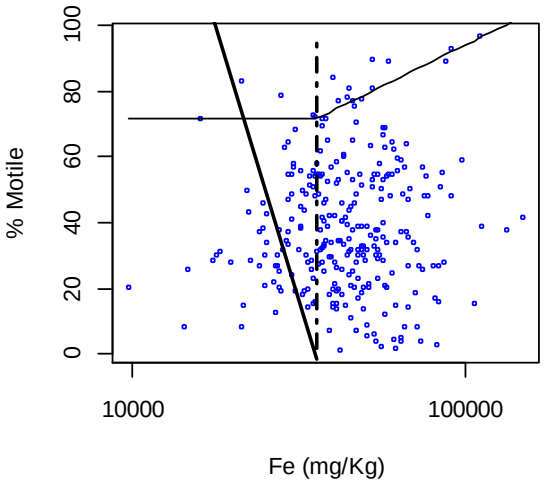

% Motile
0
20
40
60
80
100
10
100
1000
Cu (mg/Kg)

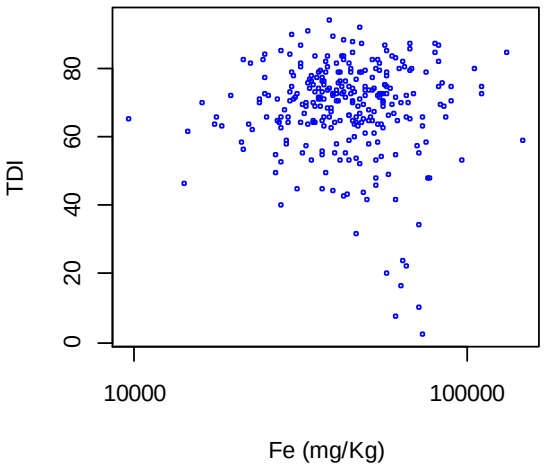

TDI
0
20
40
60
80
10
100
1000
Cu (mg/Kg)

Iron

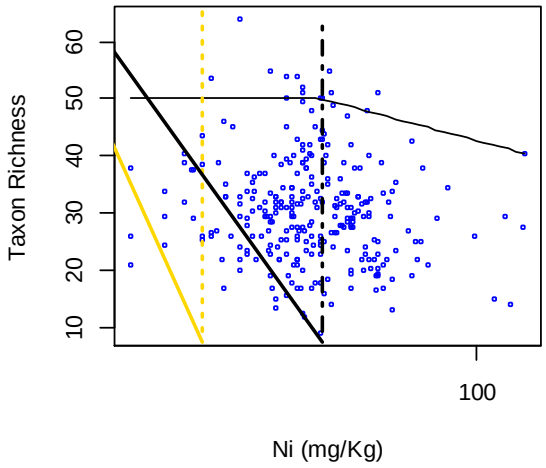

Taxon Richness
10
20
30
40
50
60
10000
100000
Fe (mg/Kg)

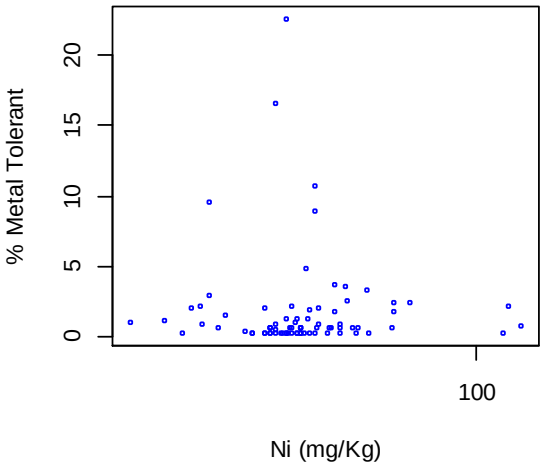

% Metal Tolerant
0
5
10
15
20
10000
100000
Fe (mg/Kg)

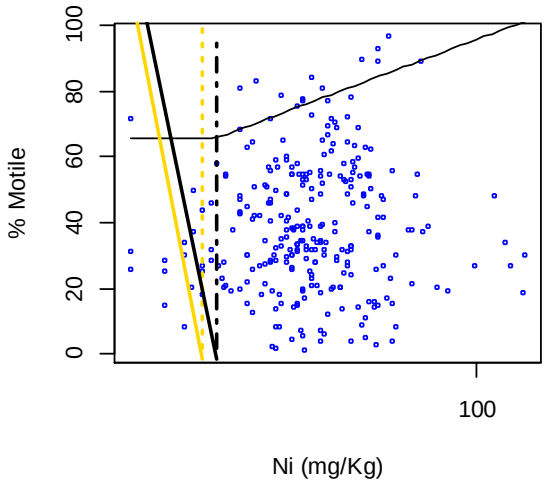

% Motile
0
20
40
60
80
100
10000
100000
Fe (mg/Kg)

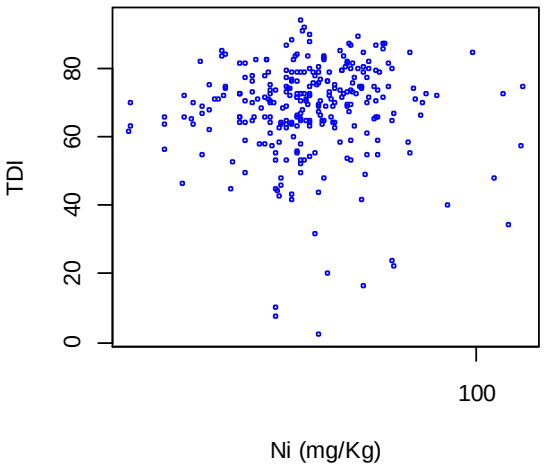

TDI
0
20
40
60
80
10000
100000
Fe (mg/Kg)

Nickel

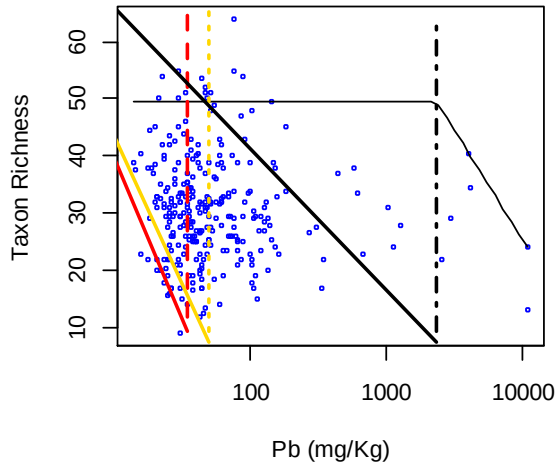

Taxon Richness
10
20
30
40
50
60
100
Ni (mg/Kg)

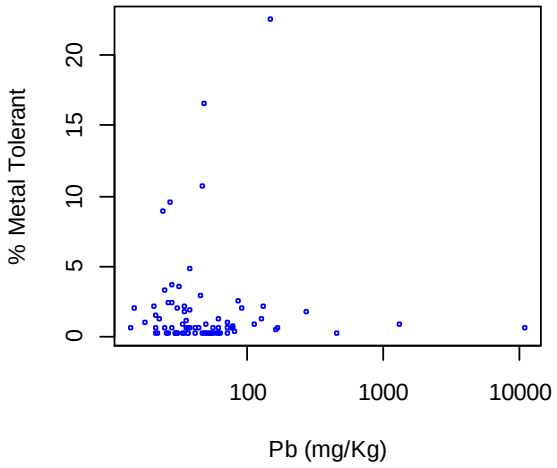

% Metal Tolerant
0
5
10
15
20
100
Ni (mg/Kg)

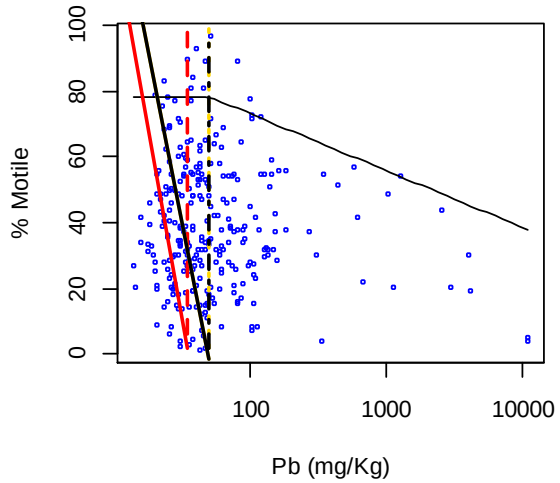

% Motile
0
20
40
60
80
100
100
Ni (mg/Kg)

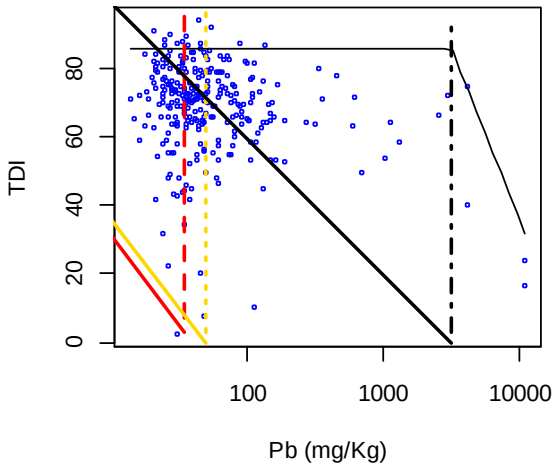

TDI
0
20
40
60
80
100
Ni (mg/Kg)

Lead

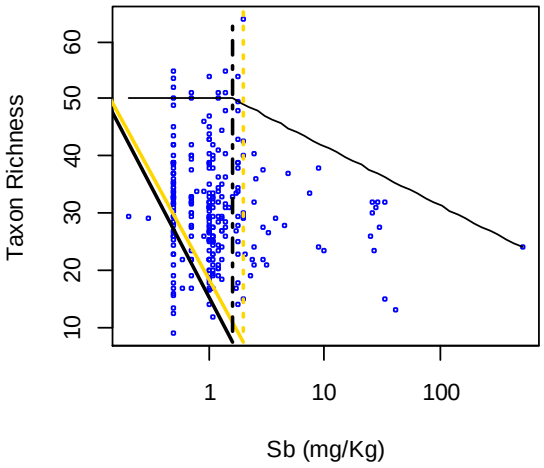

Taxon Richness
10
20
30
40
50
60
100
1000
10000
Pb (mg/Kg)

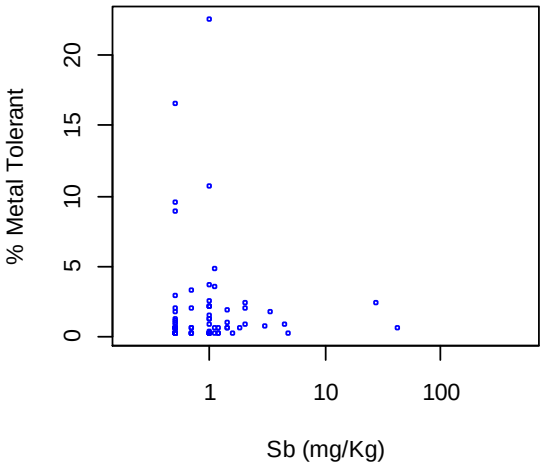

% Metal Tolerant
0
5
10
15
20
100
1000
10000
Pb (mg/Kg)

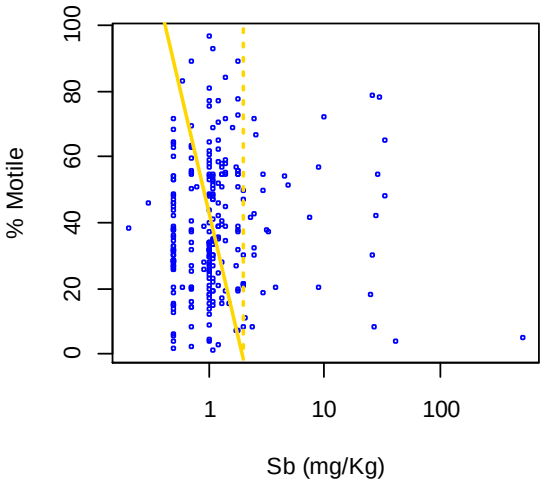

% Motile
0
20
40
60
80
100
100
1000
10000
Pb (mg/Kg)

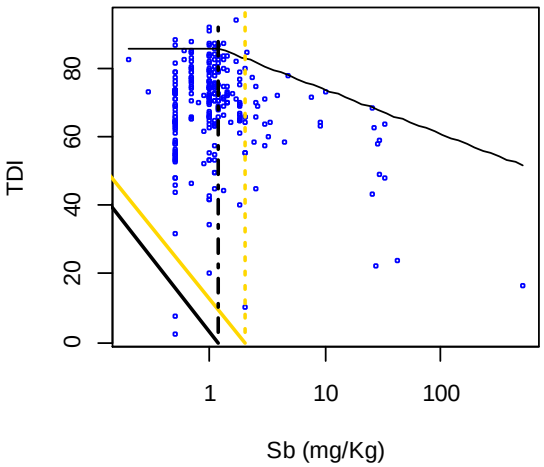

TDI
0
20
40
60
80
100
1000
10000
Pb (mg/Kg)

Antimony

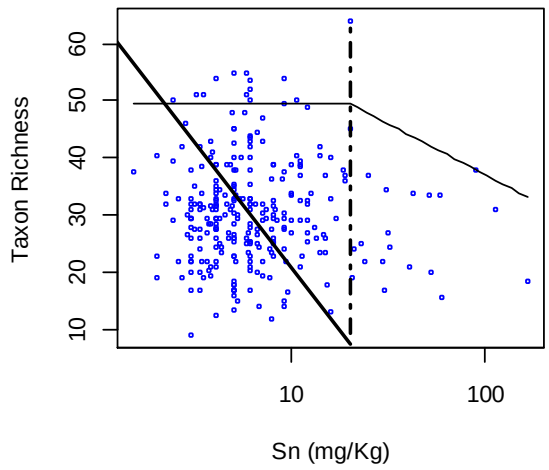

Taxon Richness
10
20
30
40
50
60
1
10
100
Sb (mg/Kg)

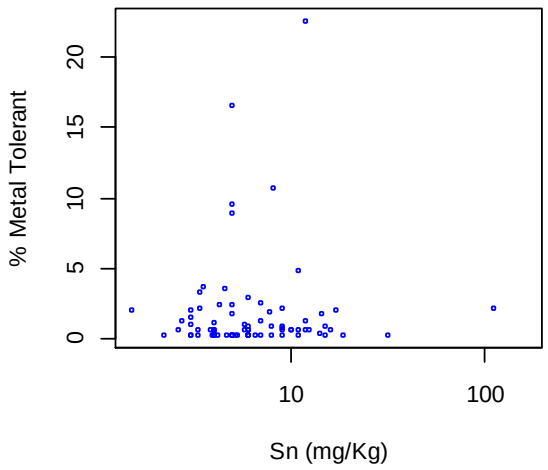

% Metal Tolerant
0
5
10
15
20
1
10
100
Sb (mg/Kg)

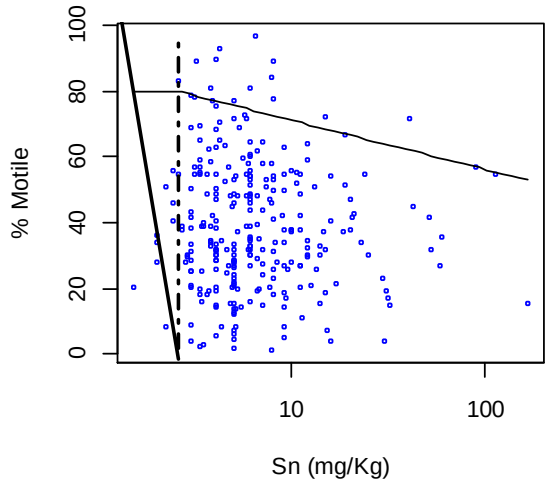

% Motile
0
20
40
60
80
100
1
10
100
Sb (mg/Kg)

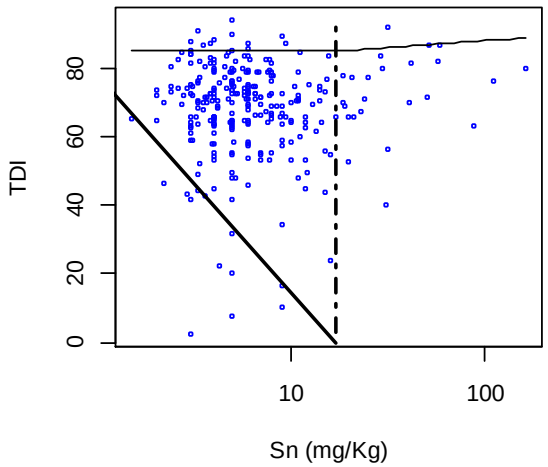

TDI
0
20
40
60
80
1
10
100
Sb (mg/Kg)

Tin

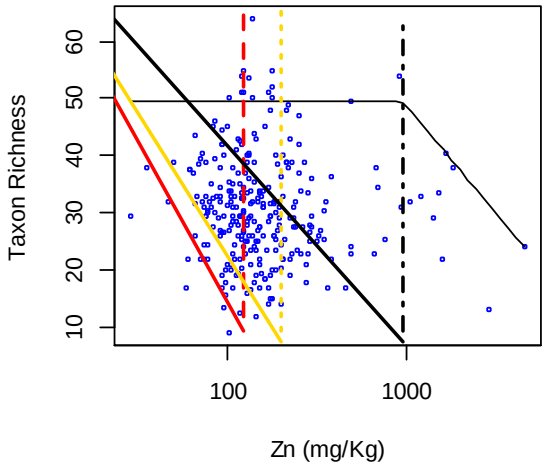

Taxon Richness
10
20
30
40
50
60
10
100
Sn (mg/Kg)

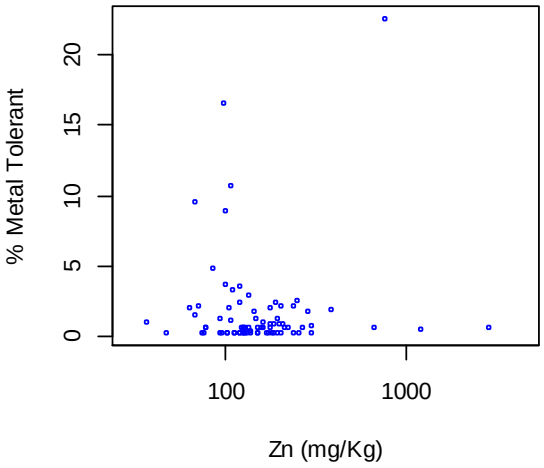

% Metal Tolerant
0
5
10
15
20
10
100
Sn (mg/Kg)

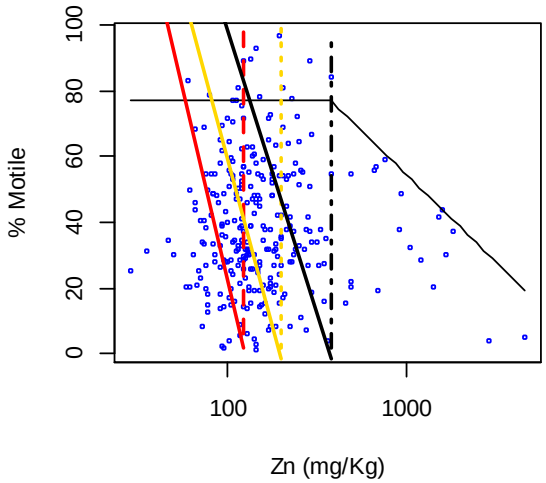

% Motile
0
20
40
60
80
100
10
100
Sn (mg/Kg)

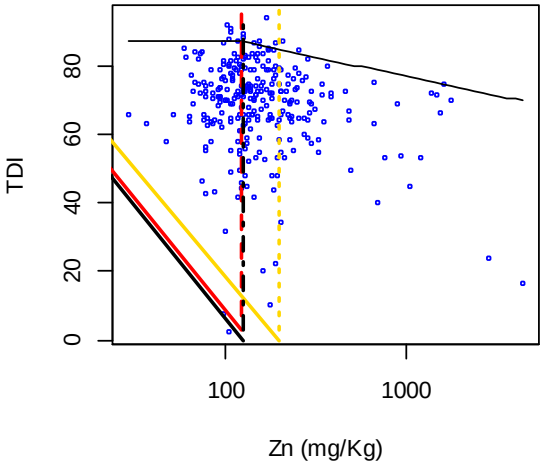

TDI
0
20
40
60
80
10
100
Sn (mg/Kg)

Zinc


Taxon Richness
10
20
30
40
50
60
100
1000
Zn (mg/Kg)


% Metal Tolerant
0
5
10
15
20
100
1000
Zn (mg/Kg)

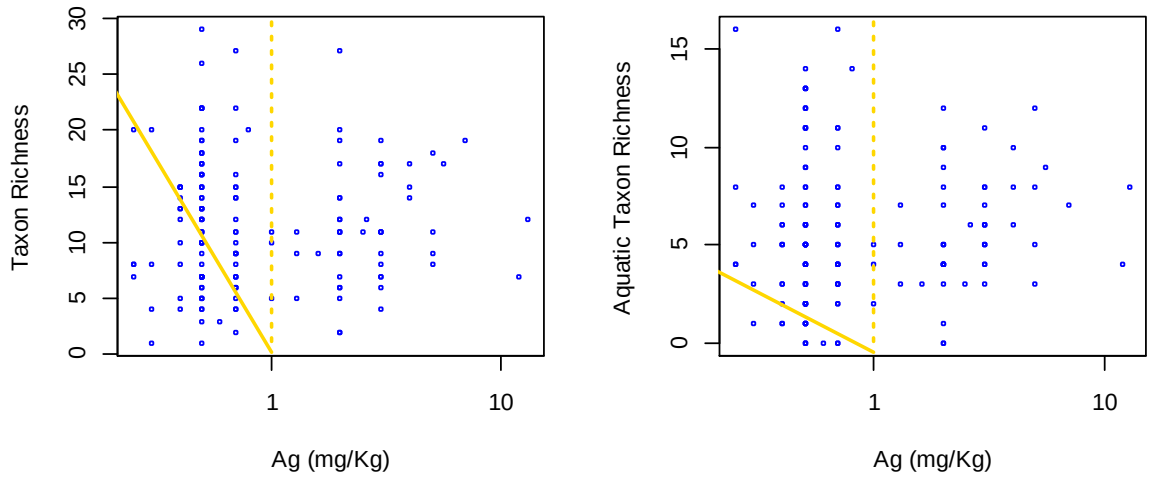

% Motile
0
20
40
60
80
100
100
1000
Zn (mg/Kg)

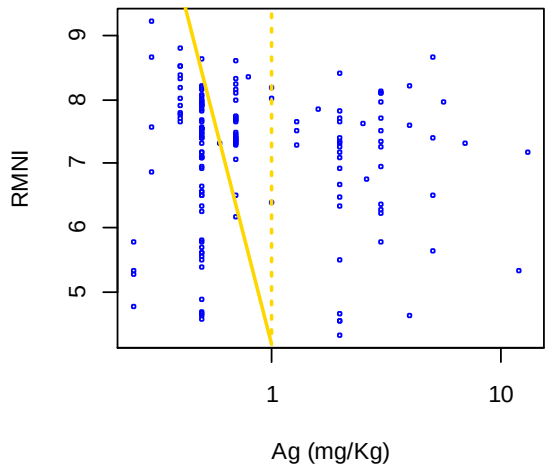

TDI
0
20
40
60
80
100
1000
Zn (mg/Kg)

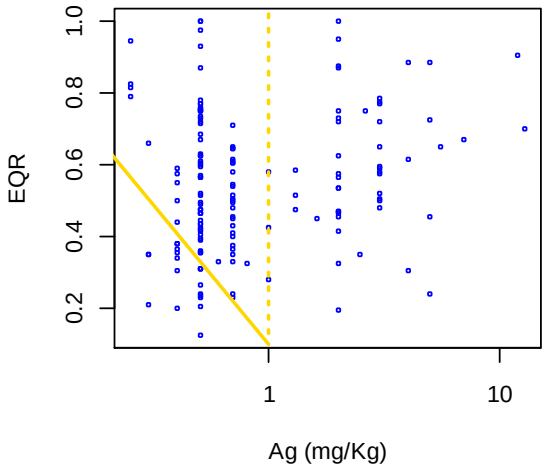
iii) Macrophytes

Plant Richness (all taxa), No. Aquatic Taxa, River Macrophyte Nutrient Index, EQR

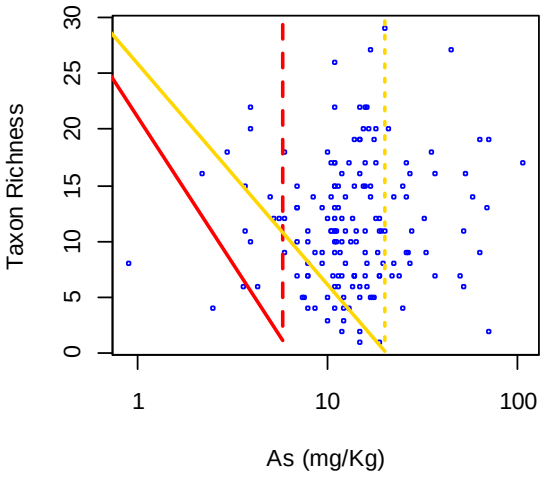
Silver

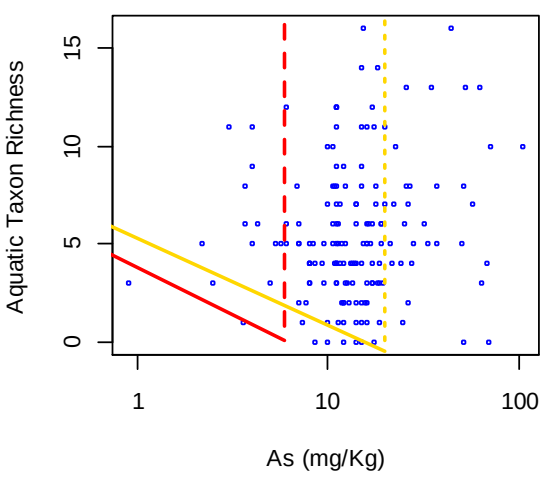


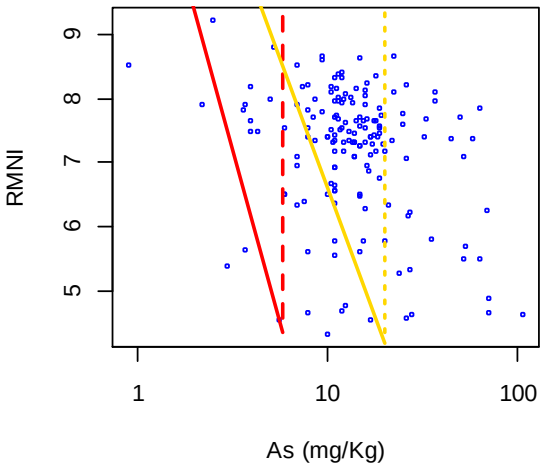


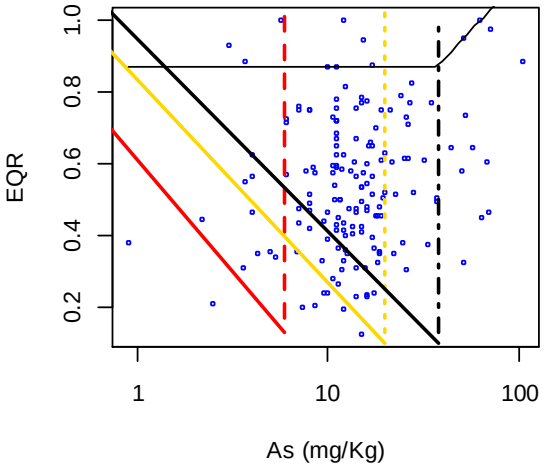


Arsenic

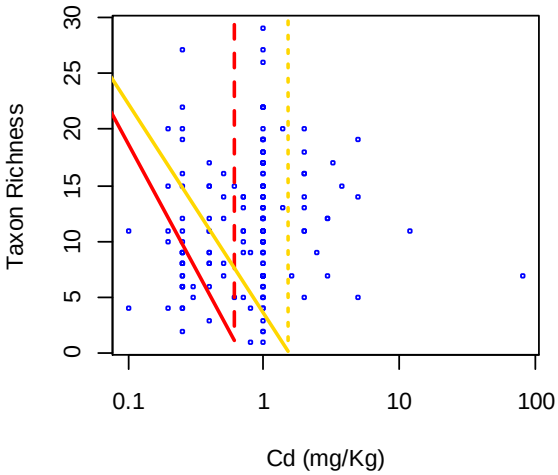

Taxon Richness
0
5
10
15
20
25
30
1
10
100
As (mg/Kg)

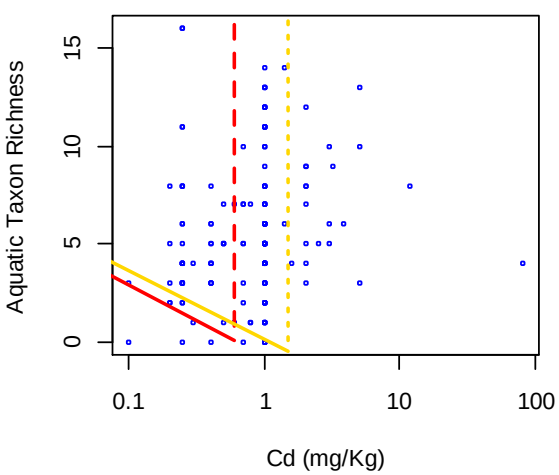

Aquatic Taxon Richness
0
5
10
15
1
10
100
As (mg/Kg)

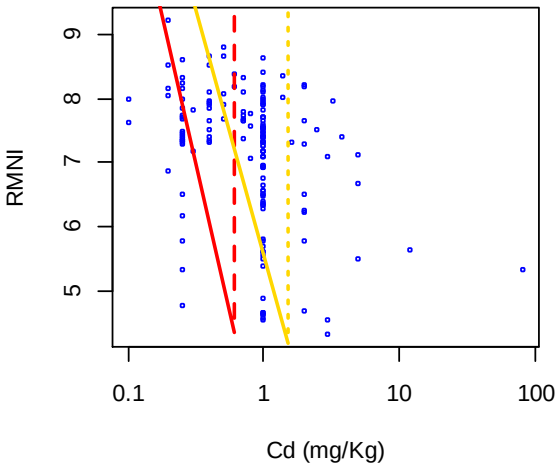

RMNI
5
6
7
8
9
1
10
100
As (mg/Kg)

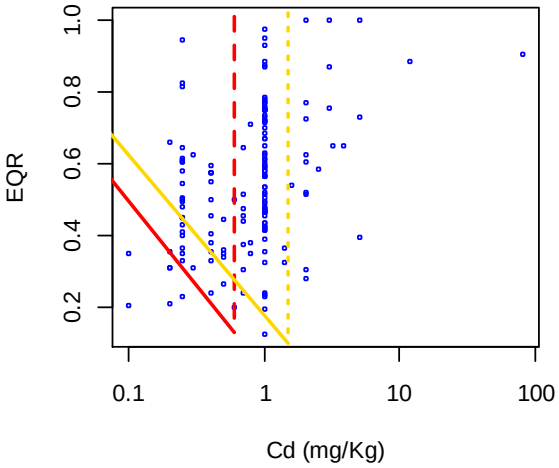

EQR
0.2
0.4
0.6
0.8
1.0
1
10
100
As (mg/Kg)

Cadmium

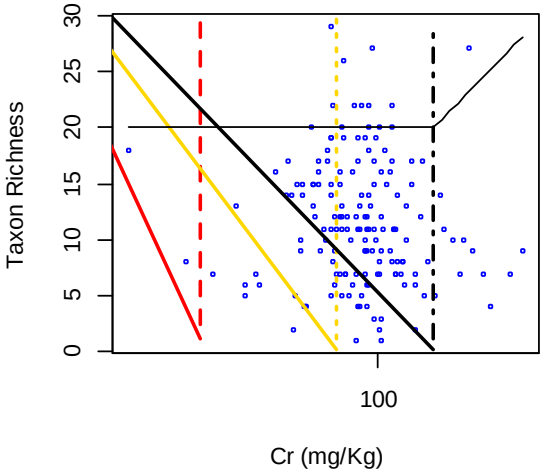

Taxon Richness
0
5
10
15
20
25
30
0.1
1
10
100
Cd (mg/Kg)

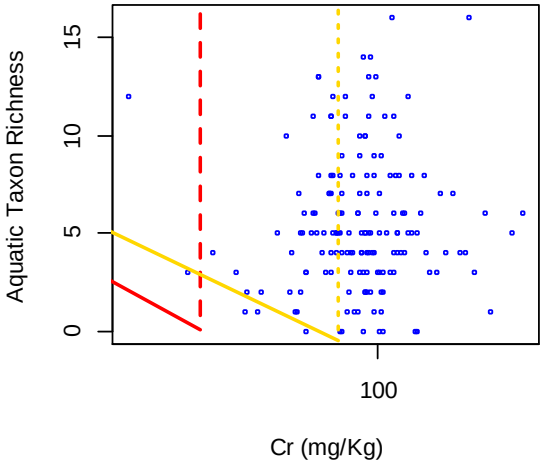

Aquatic Taxon Richness
0
5
10
15
0.1
1
10
100
Cd (mg/Kg)

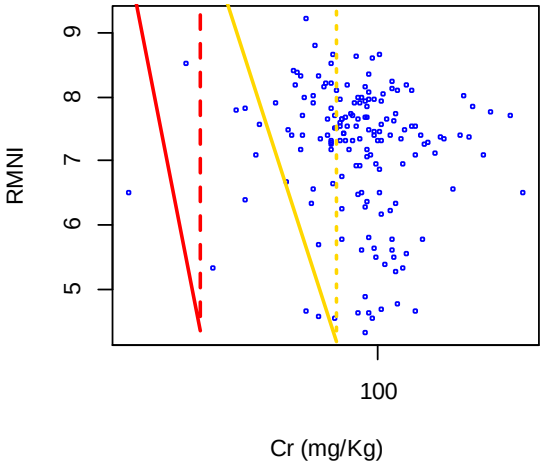

RMNI
5
6
7
8
9
0.1
1
10
100
Cd (mg/Kg)

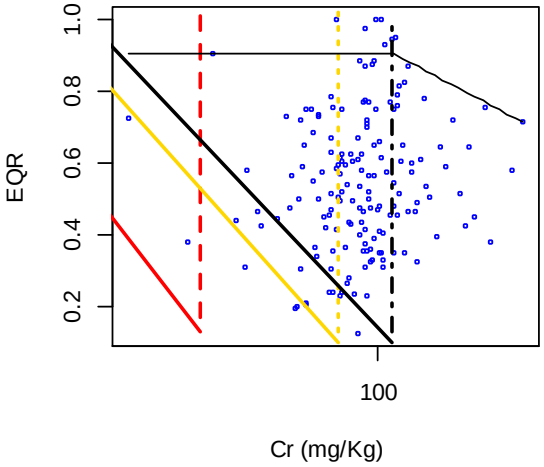

EQR
0.2
0.4
0.6
0.8
1.0
0.1
1
10
100
Cd (mg/Kg)

Chromium

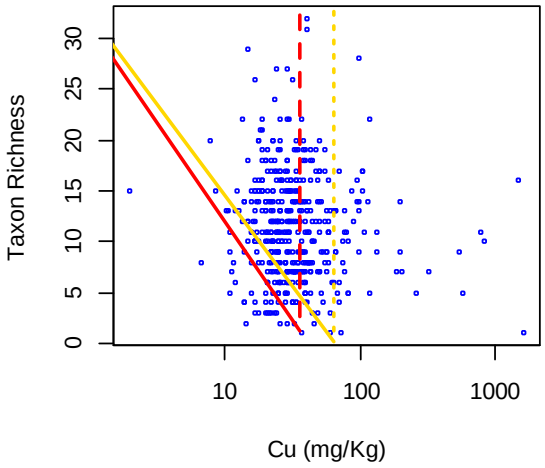

Taxon Richness
0
5
10
15
20
25
30
100
Cr (mg/Kg)

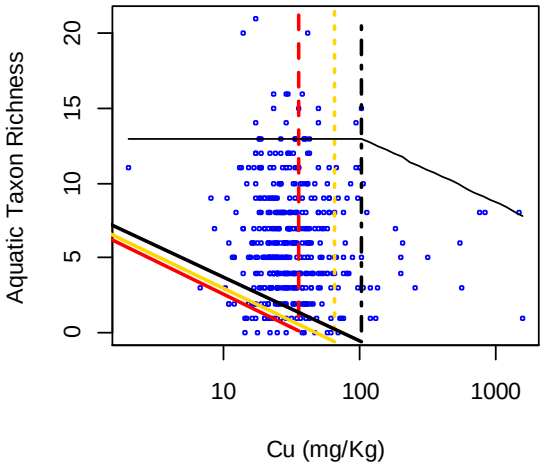

Aquatic Taxon Richness
0
5
10
15
100
Cr (mg/Kg)

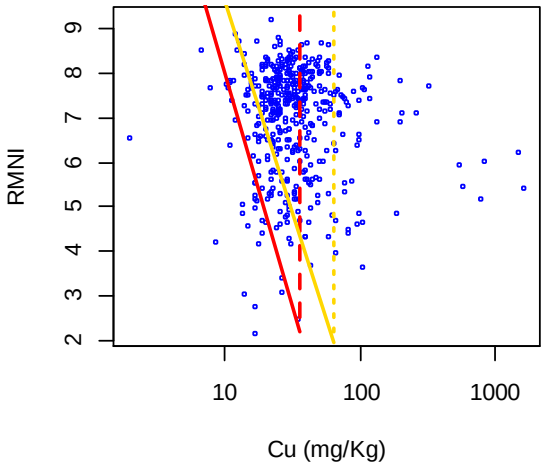

RMNI
5
6
7
8
9
100
Cr (mg/Kg)

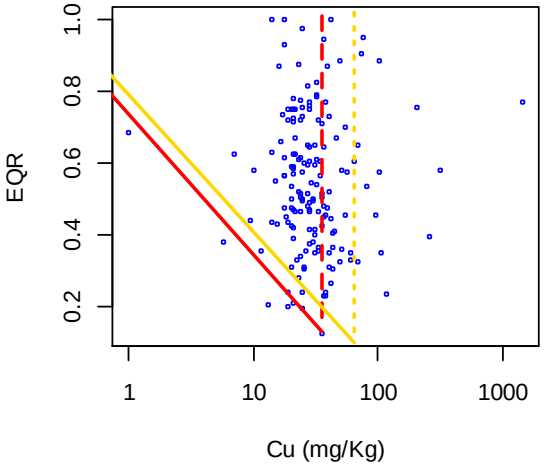

EQR
0.2
0.4
0.6
0.8
1.0
100
Cr (mg/Kg)

Copper

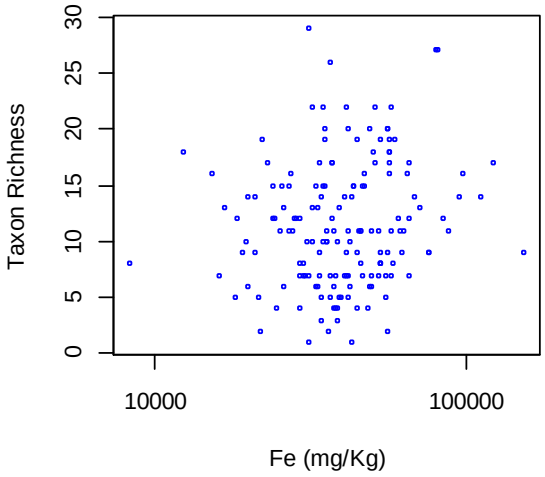

Taxon Richness
0
5
10
15
20
25
30
10
100
1000
Cu (mg/Kg)

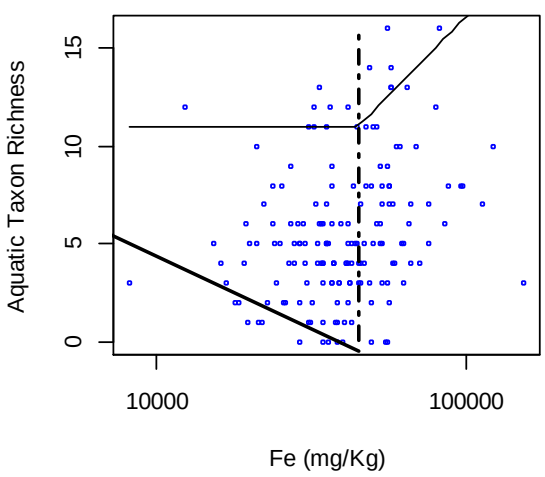

Aquatic Taxon Richness
0
5
10
15
20
10
100
1000
Cu (mg/Kg)

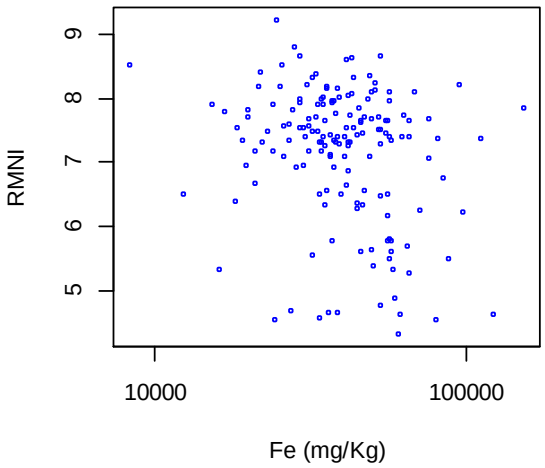

RMNI
2
3
4
5
6
7
8
9
10
100
1000
Cu (mg/Kg)

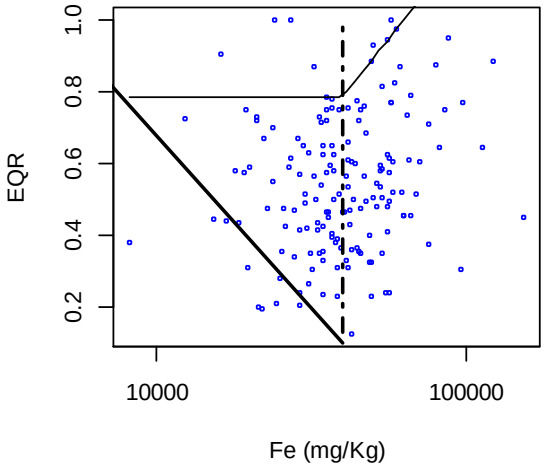

EQR
0.2
0.4
0.6
0.8
1.0
1
10
100
1000
Cu (mg/Kg)

Iron

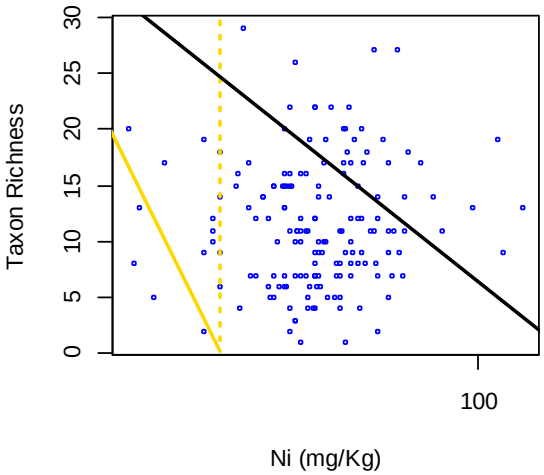

Taxon Richness
0
5
10
15
20
25
30
10000
100000
Fe (mg/Kg)

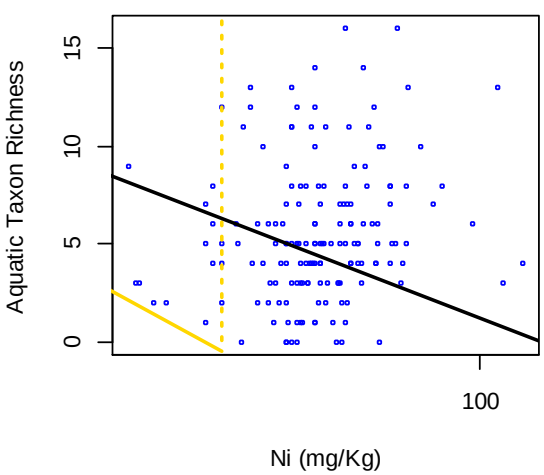

Aquatic Taxon Richness
0
5
10
15
10000
100000
Fe (mg/Kg)

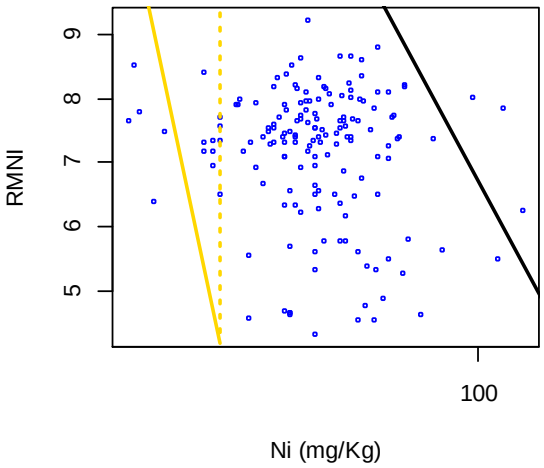

RMNI
5
6
7
8
9
10000
100000
Fe (mg/Kg)

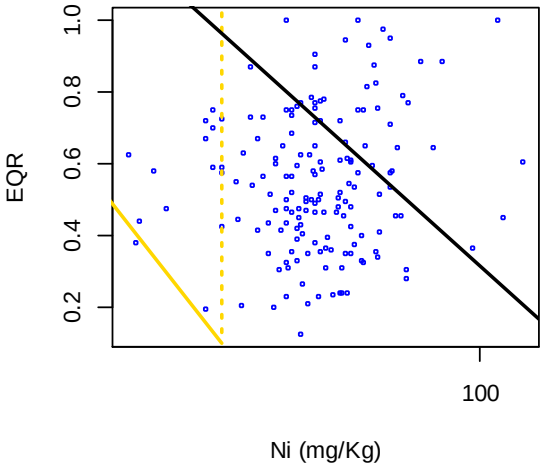

EQR
0.2
0.4
0.6
0.8
1.0
10000
100000
Fe (mg/Kg)

Nickel

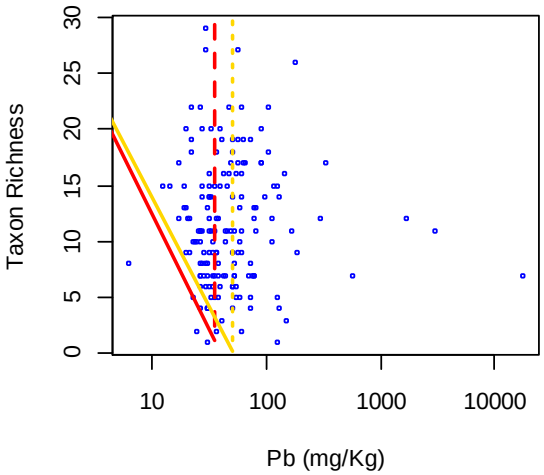

Taxon Richness
0
5
10
15
20
25
30
100
Ni (mg/Kg)

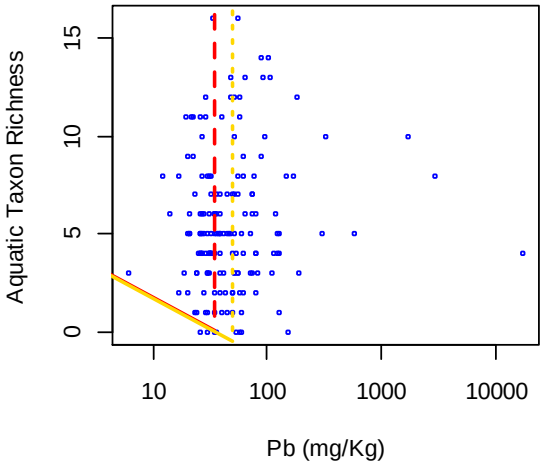

Aquatic Taxon Richness
0
5
10
15
100
Ni (mg/Kg)

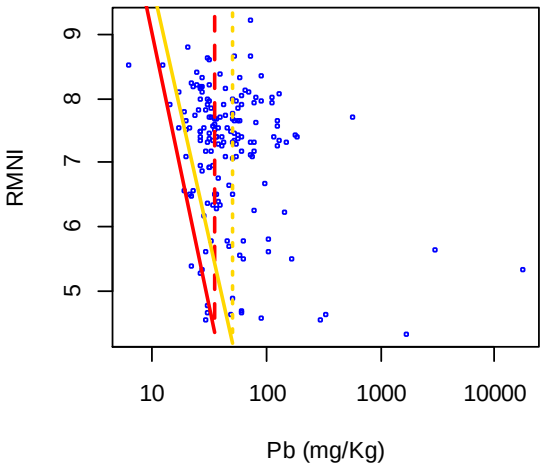

RMNI
5
6
7
8
9
100
Ni (mg/Kg)

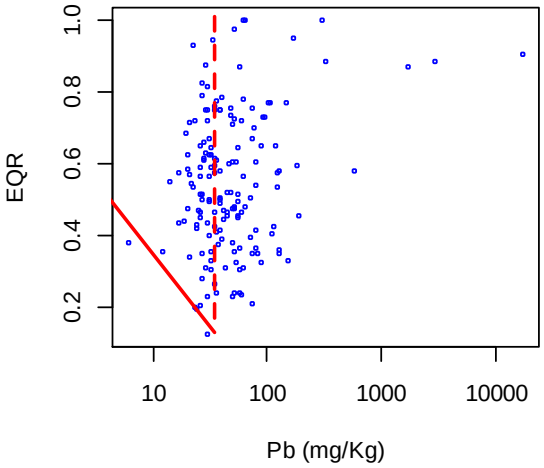

EQR
0.2
0.4
0.6
0.8
1.0
100
Ni (mg/Kg)

Lead

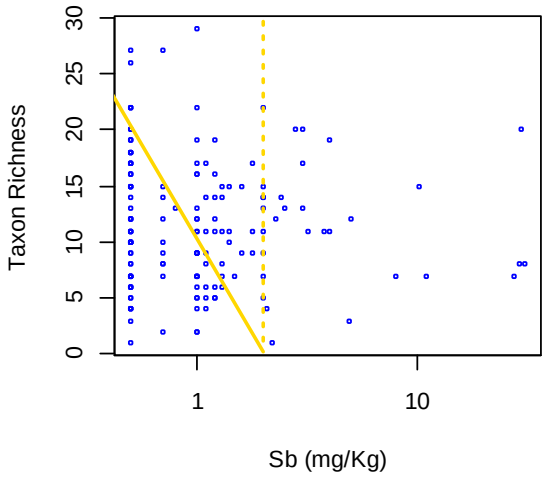

Taxon Richness
0
5
10
15
20
25
30
10
100
1000
10000
Pb (mg/Kg)

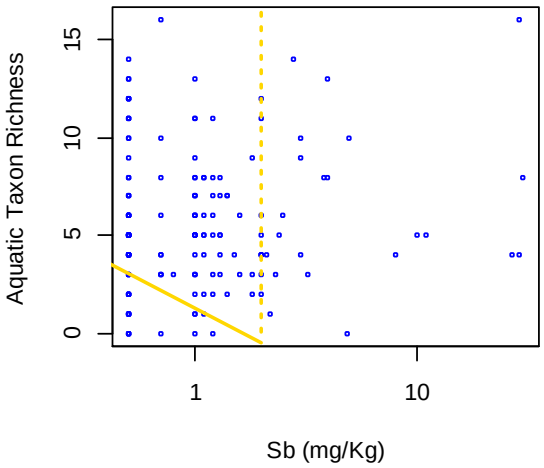

Aquatic Taxon Richness
0
5
10
15
10
100
1000
10000
Pb (mg/Kg)

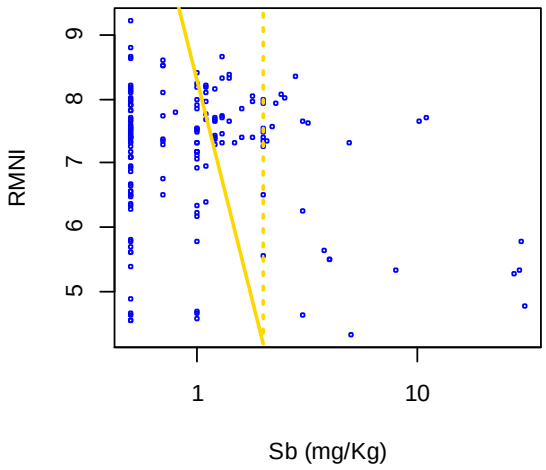

RMNI
5
6
7
8
9
10
100
1000
10000
Pb (mg/Kg)

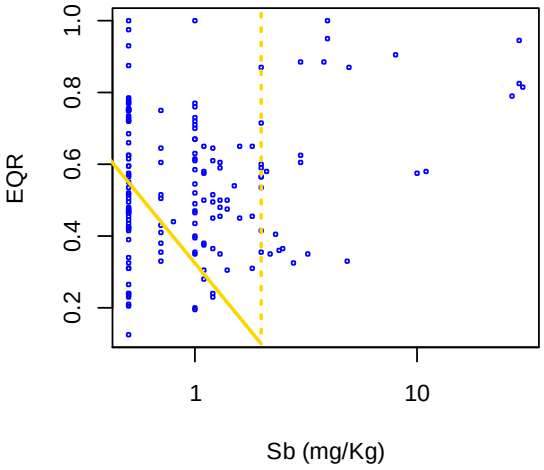

EQR
0.2
0.4
0.6
0.8
1.0
10
100
1000
10000
Pb (mg/Kg)

Antimony

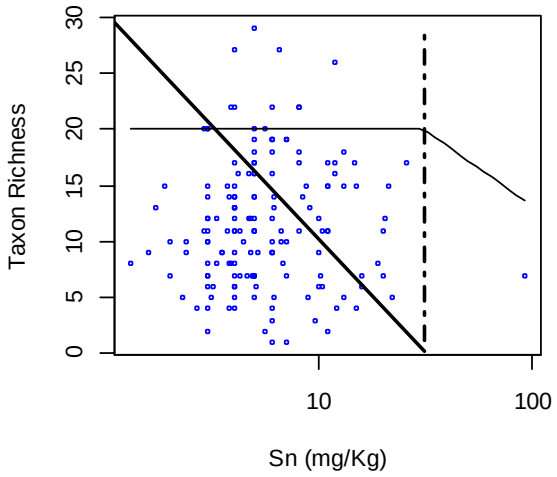

Taxon Richness
0
5
10
15
20
25
30
1
10
Sb (mg/Kg)

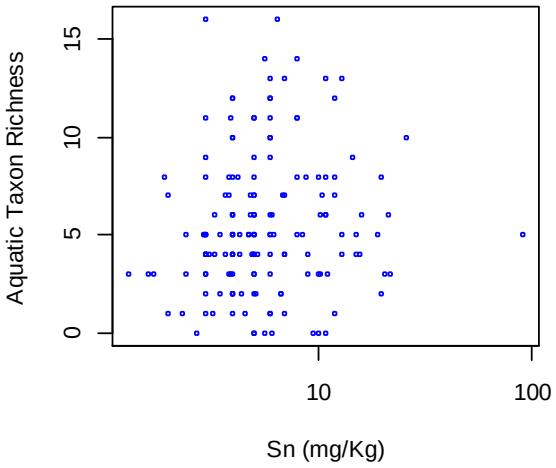

Aquatic Taxon Richness
0
5
10
15
1
10
Sb (mg/Kg)

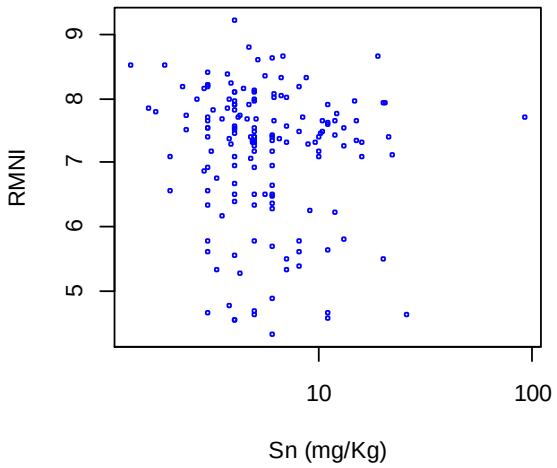

RMNI
5
6
7
8
9
1
10
Sb (mg/Kg)

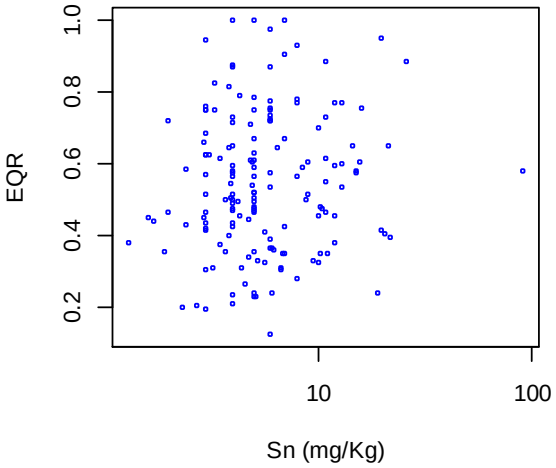

EQR
0.2
0.4
0.6
0.8
1.0
1
10
Sb (mg/Kg)

Tin

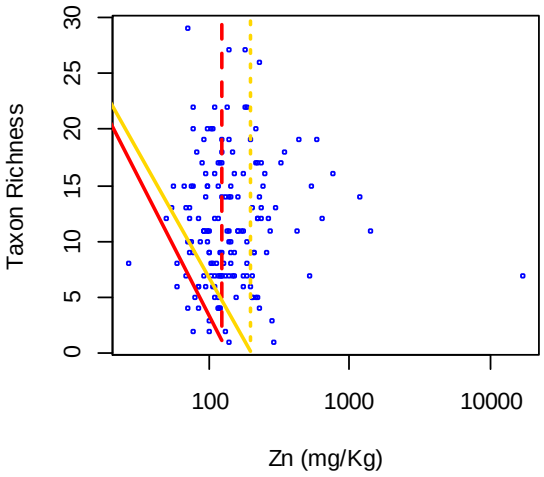

Taxon Richness
0
5
10
15
20
25
30
10
100
Sn (mg/Kg)

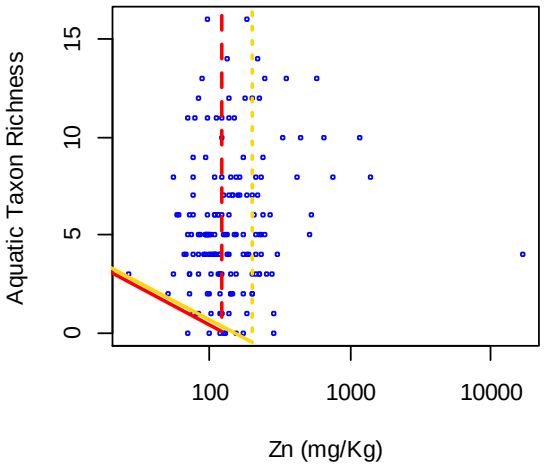

Aquatic Taxon Richness
0
5
10
15
10
100
Sn (mg/Kg)

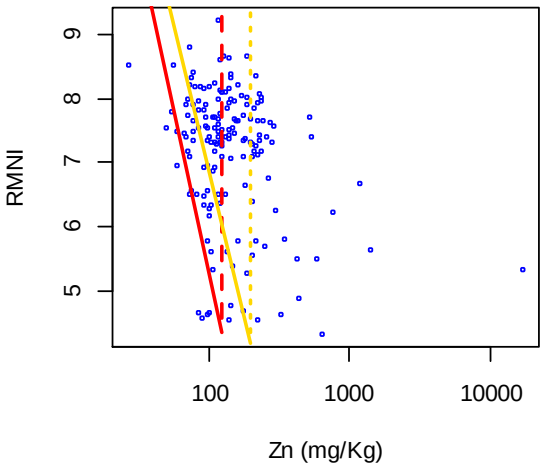

RMNI
5
6
7
8
9
10
100
Sn (mg/Kg)

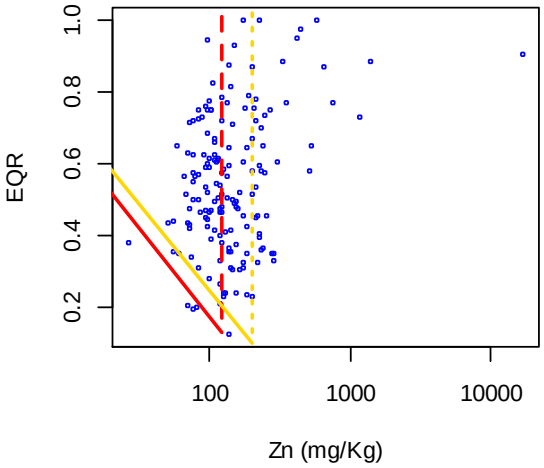

EQR
0.2
0.4
0.6
0.8
1.0
10
100
Sn (mg/Kg)

Zinc


Taxon Richness
0
5
10
15
20
25
30
100
1000
10000
Zn (mg/Kg)

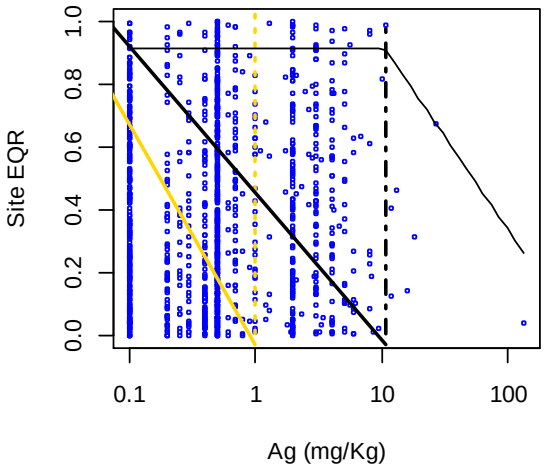

Aquatic Taxon Richness
0
5
10
15
100
1000
10000
Zn (mg/Kg)

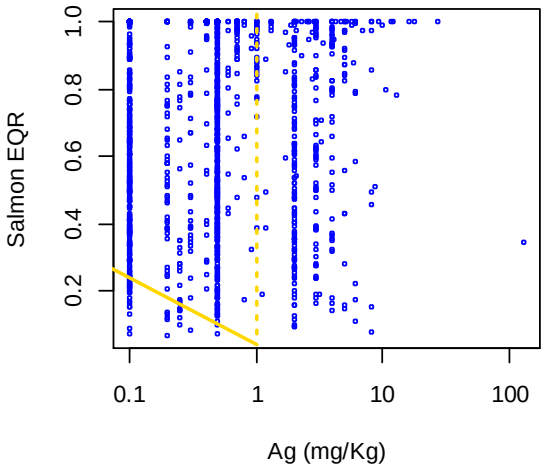

RMNI
5
6
7
8
9
100
1000
10000
Zn (mg/Kg)

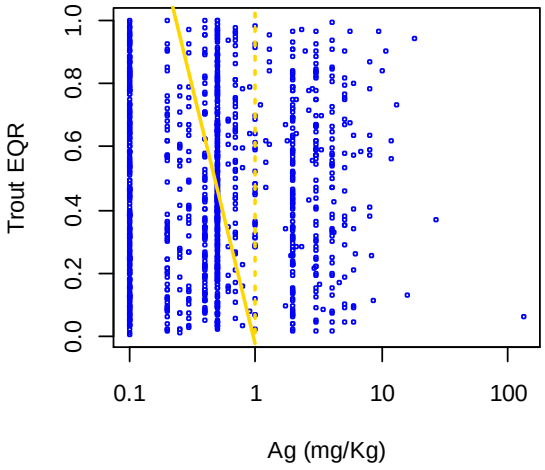

EQR
0.2
0.4
0.6
0.8
1.0
100
1000
10000
Zn (mg/Kg)

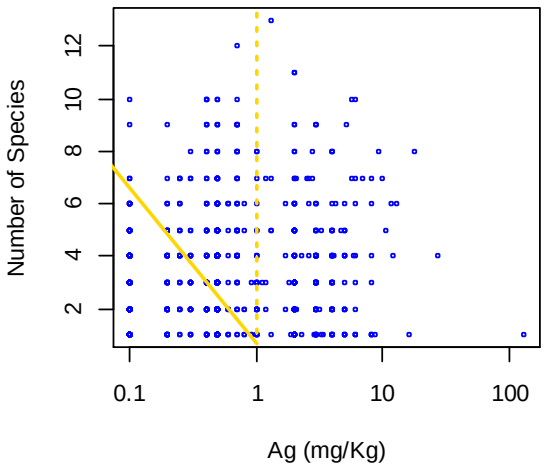
iv) Fish

Site EQR, Salmon EQR, Trout EQR, Number of Species

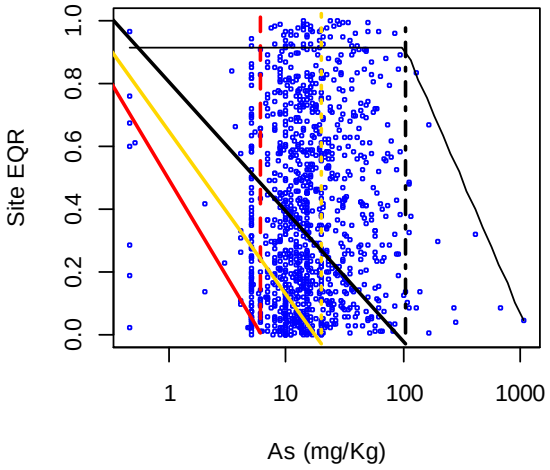


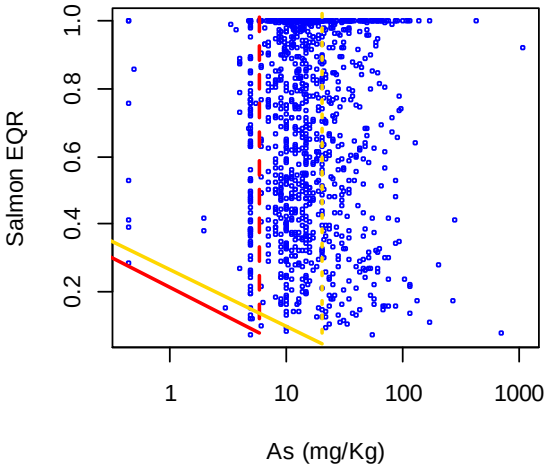


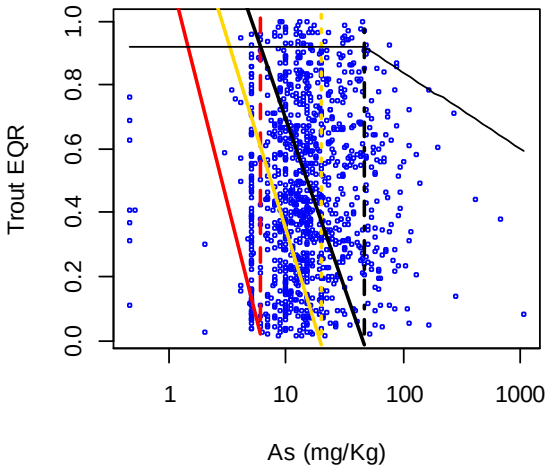


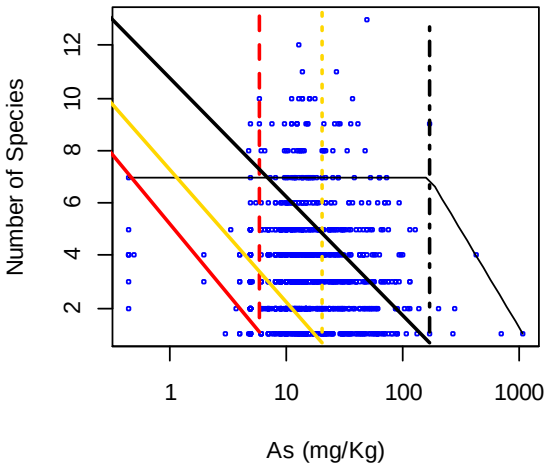


Arsenic

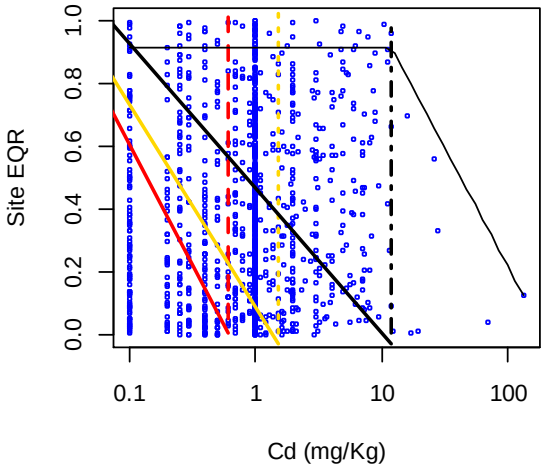

Site EQR
0.0
0.2
0.4
0.6
0.8
1.0
1
10
100
1000
As (mg/Kg)

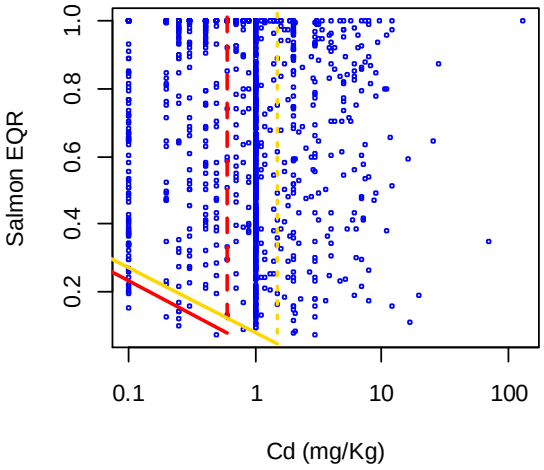

Salmon EQR
0.2
0.4
0.6
0.8
1.0
1
10
100
1000
As (mg/Kg)

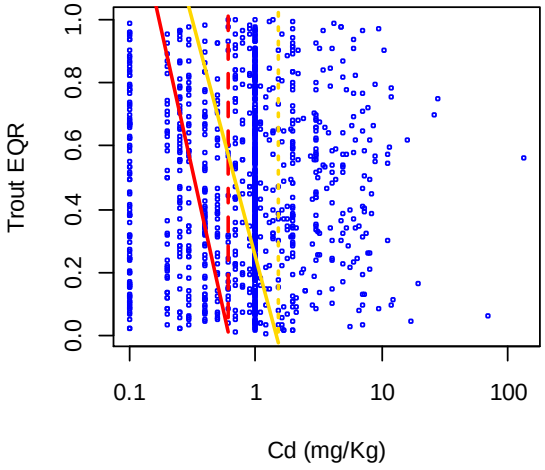

Trout EQR
0.0
0.2
0.4
0.6
0.8
1.0
1
10
100
1000
As (mg/Kg)

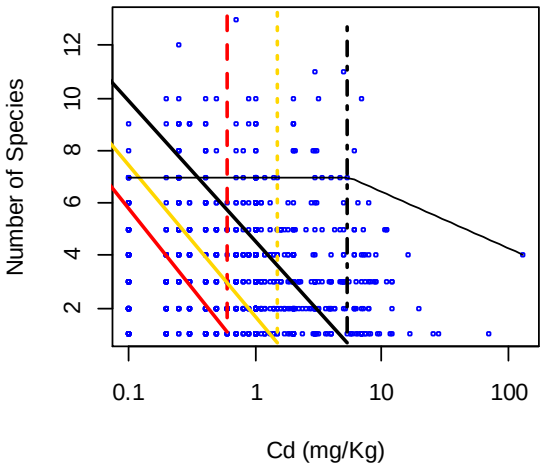

Number of Species
2
4
6
8
10
12
1
10
100
1000
As (mg/Kg)

Cadmium

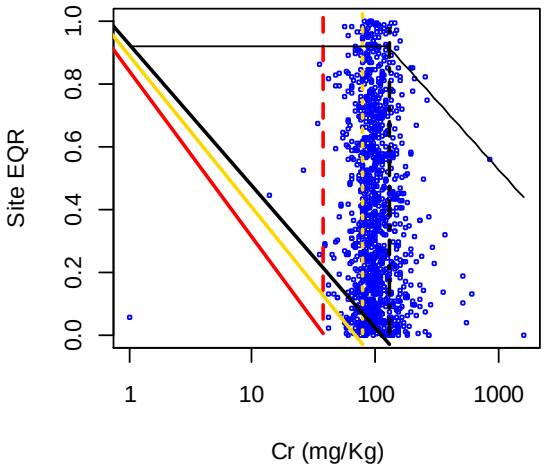

Site EQR
0.0
0.2
0.4
0.6
0.8
1.0
0.1
1
10
100
Cd (mg/Kg)

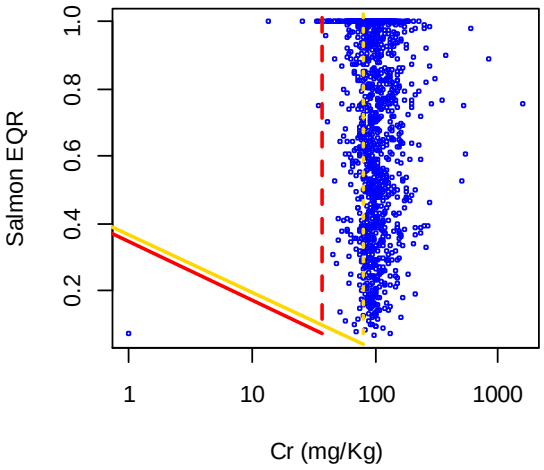

Salmon EQR
0.2
0.4
0.6
0.8
1.0
0.1
1
10
100
Cd (mg/Kg)

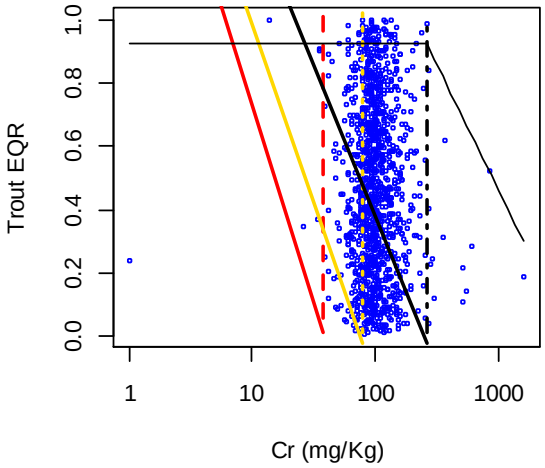

Trout EQR
0.0
0.2
0.4
0.6
0.8
1.0
0.1
1
10
100
Cd (mg/Kg)

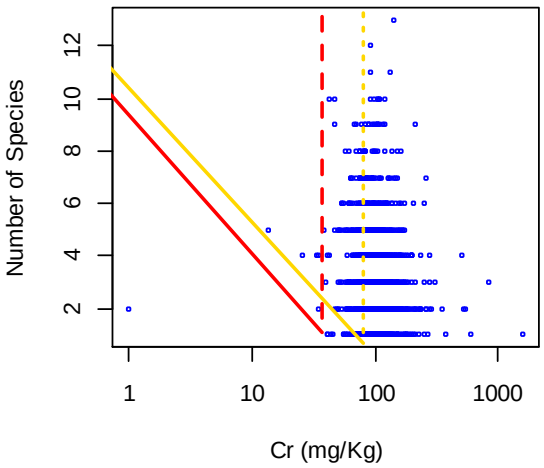

Number of Species
2
4
6
8
10
12
0.1
1
10
100
Cd (mg/Kg)

Chromium

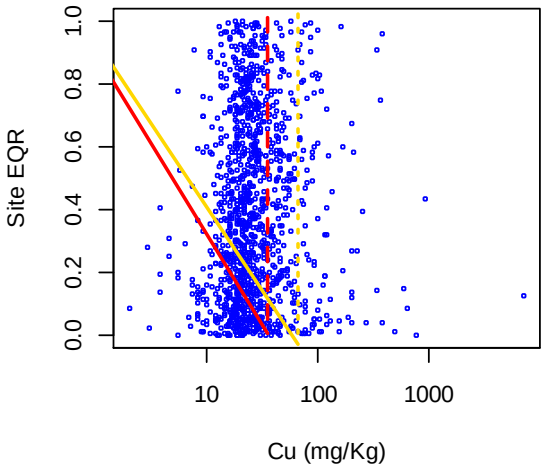

Site EQR
0.0
0.2
0.4
0.6
0.8
1.0
1
10
100
1000
Cr (mg/Kg)

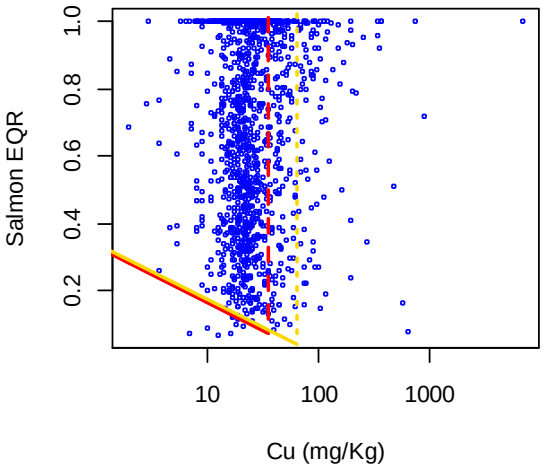

Salmon EQR
0.2
0.4
0.6
0.8
1.0
1
10
100
1000
Cr (mg/Kg)

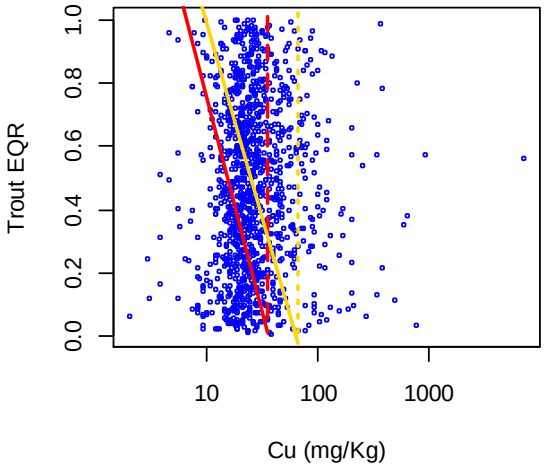

Trout EQR
0.0
0.2
0.4
0.6
0.8
1.0
1
10
100
1000
Cr (mg/Kg)

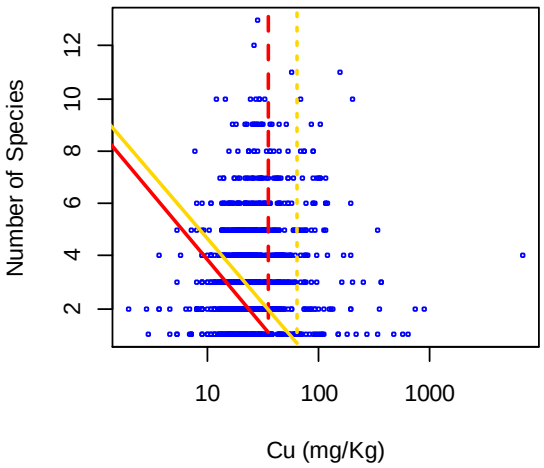

Number of Species
2
4
6
8
10
12
1
10
100
1000
Cr (mg/Kg)

Copper

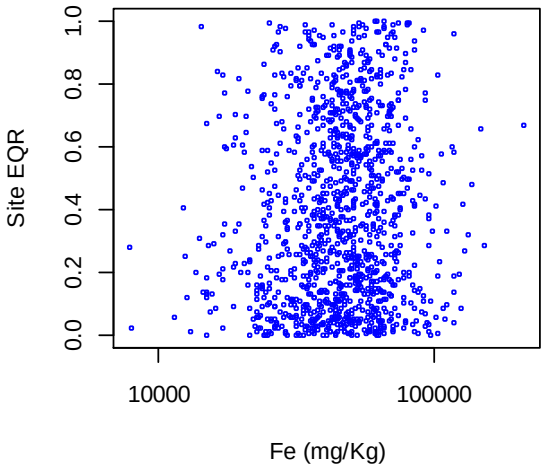

Site EQR
0.0
0.2
0.4
0.6
0.8
1.0
10
100
1000
Cu (mg/Kg)

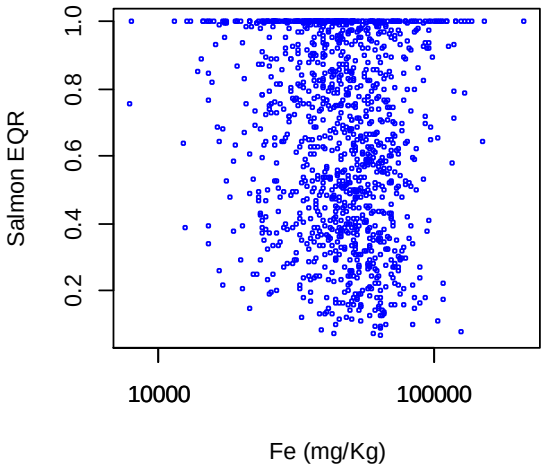

Salmon EQR
0.2
0.4
0.6
0.8
1.0
10
100
1000
Cu (mg/Kg)

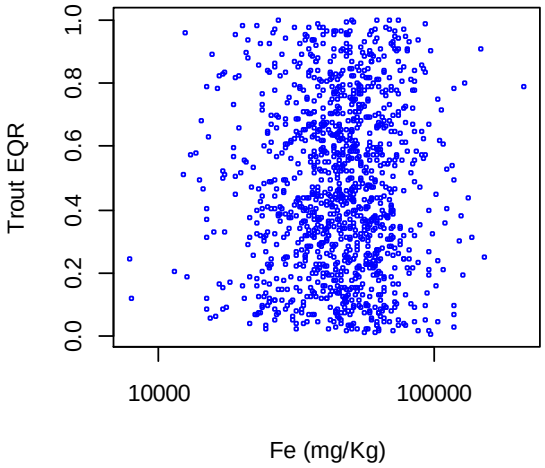

Trout EQR
0.0
0.2
0.4
0.6
0.8
1.0
10
100
1000
Cu (mg/Kg)

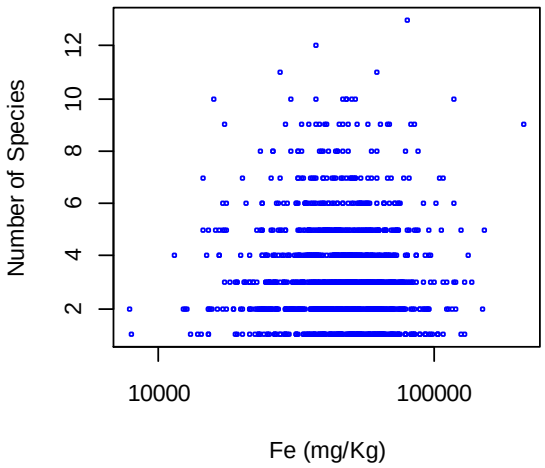

Number of Species
2
4
6
8
10
12
10
100
1000
Cu (mg/Kg)

Iron

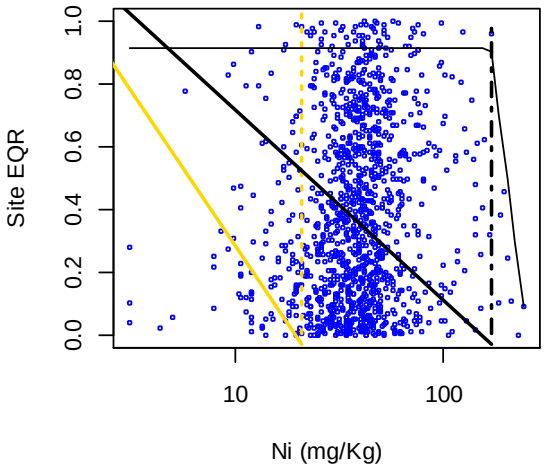

Site EQR
0.0
0.2
0.4
0.6
0.8
1.0
10000
100000
Fe (mg/Kg)

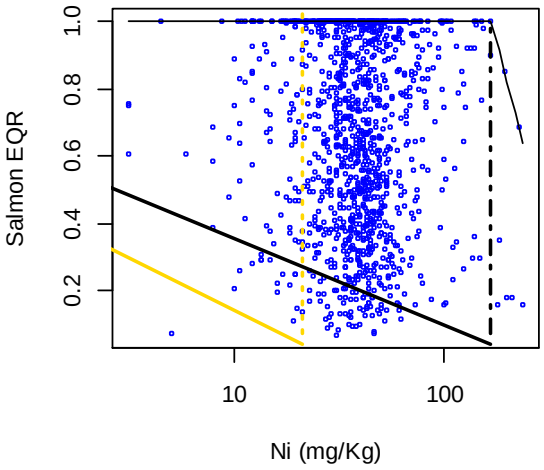

Salmon EQR
0.2
0.4
0.6
0.8
1.0
10000
100000
Fe (mg/Kg)

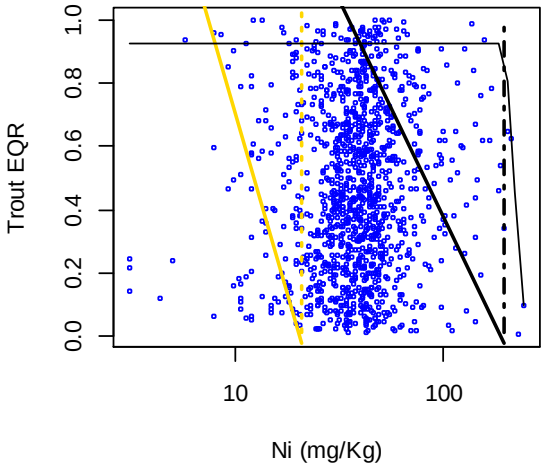

Trout EQR
0.0
0.2
0.4
0.6
0.8
1.0
10000
100000
Fe (mg/Kg)

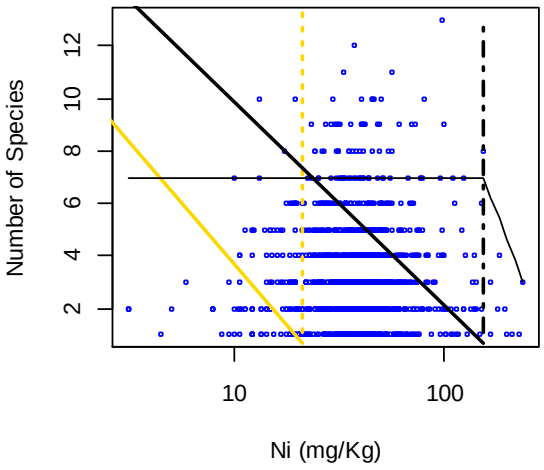

Number of Species
2
4
6
8
10
12
10000
100000
Fe (mg/Kg)

Nickel


Site EQR
0.0
0.2
0.4
0.6
0.8
1.0
10
100
Ni (mg/Kg)

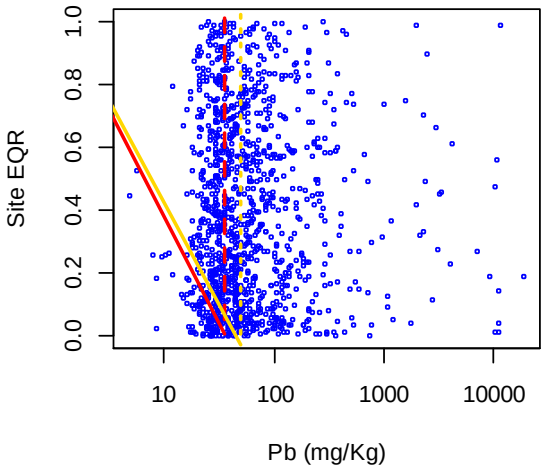

Salmon EQR
0.2
0.4
0.6
0.8
1.0
10
100
Ni (mg/Kg)

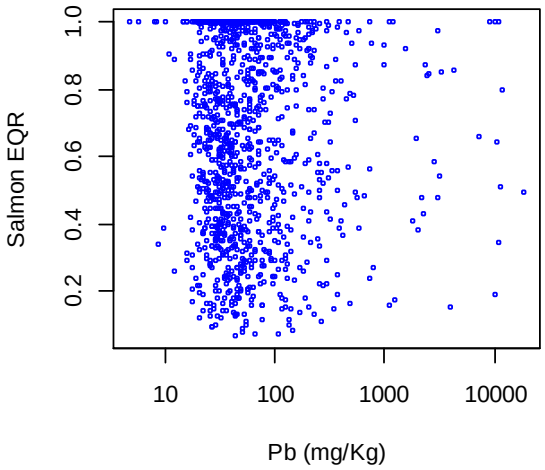

Trout EQR
0.0
0.2
0.4
0.6
0.8
1.0
10
100
Ni (mg/Kg)

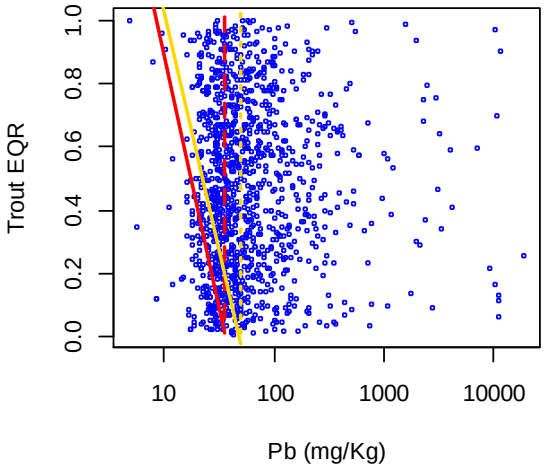

Number of Species
2
4
6
8
10
12
10
100
Ni (mg/Kg)

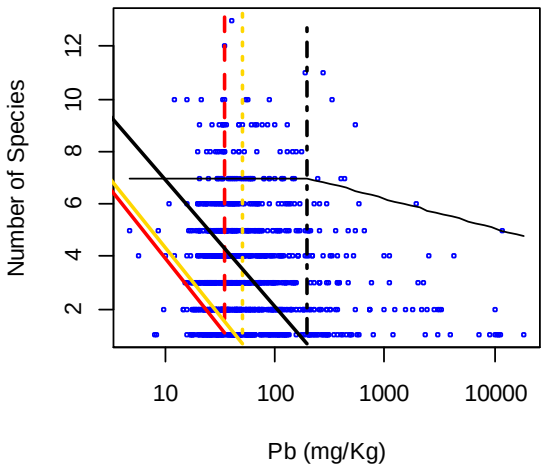
Lead

[including the Stanhope Burn]



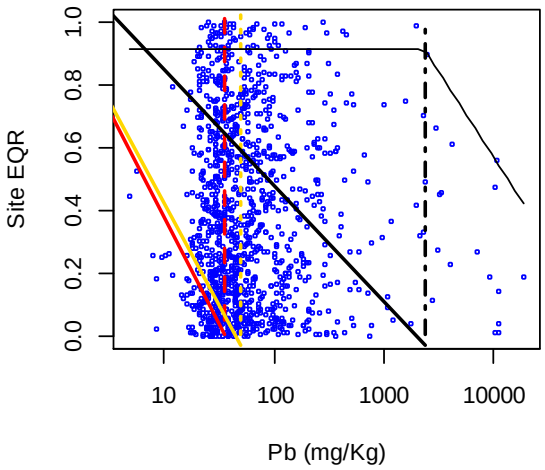


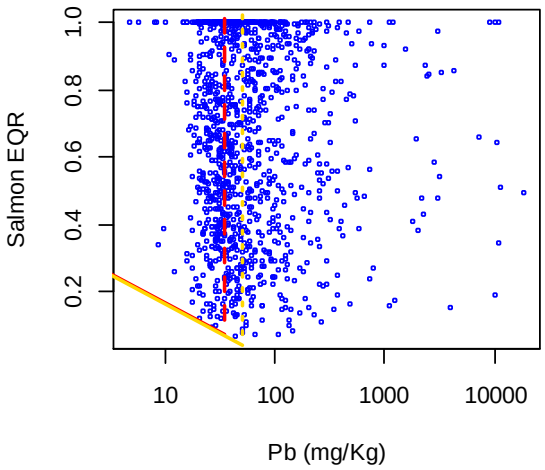


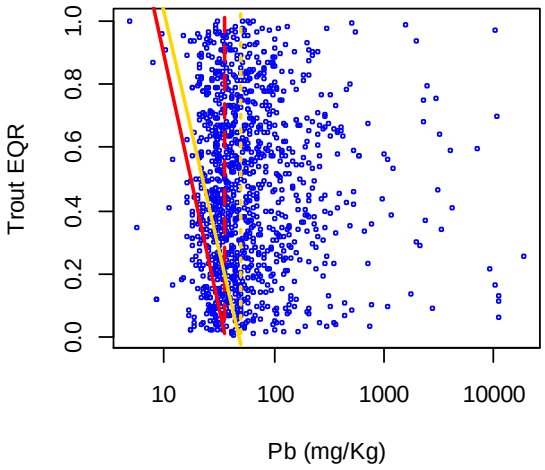


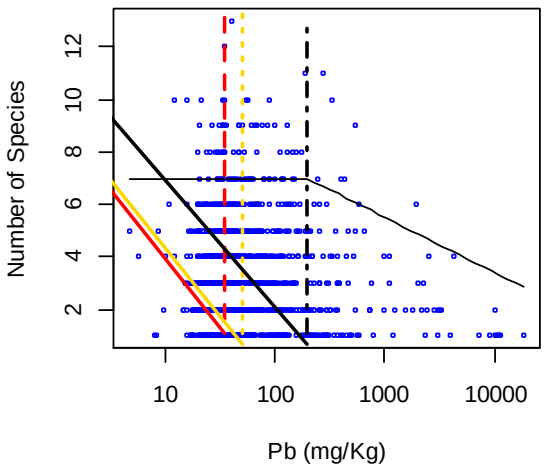
Lead

[excluding the Stanhope Burn]

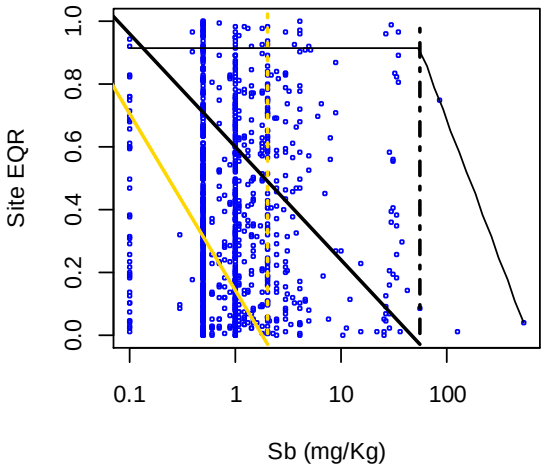








Antimony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0.4
0.6
0.8
1.0
0.1
1
10
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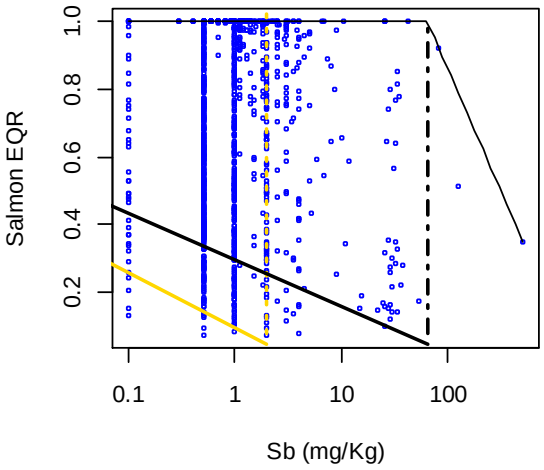
Sb (mg/Kg)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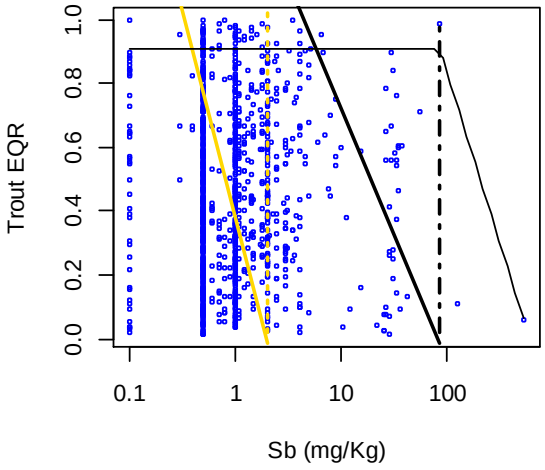
0.8
1.0
0.1
1
10
100
Sb (mg/Kg)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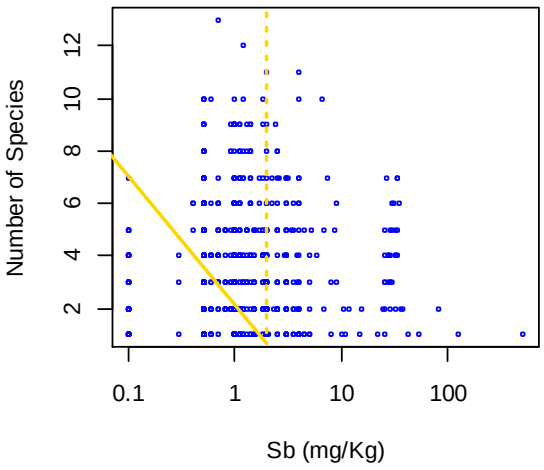
0.8
1.0
0.1
1
10
100
Sb (mg/Kg)


Number of Species
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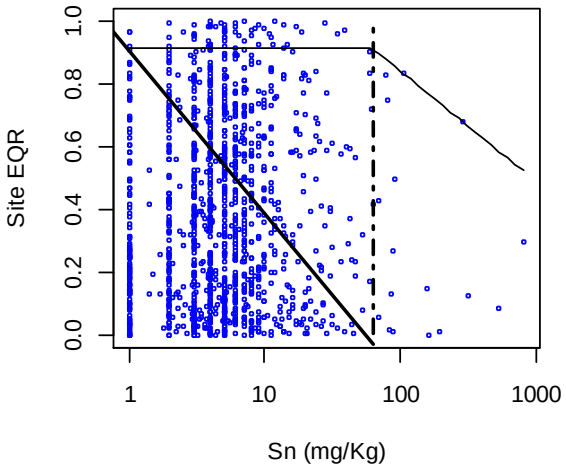
6
8
10
12
0.1
1
10
100
Sb (mg/Kg)

Tin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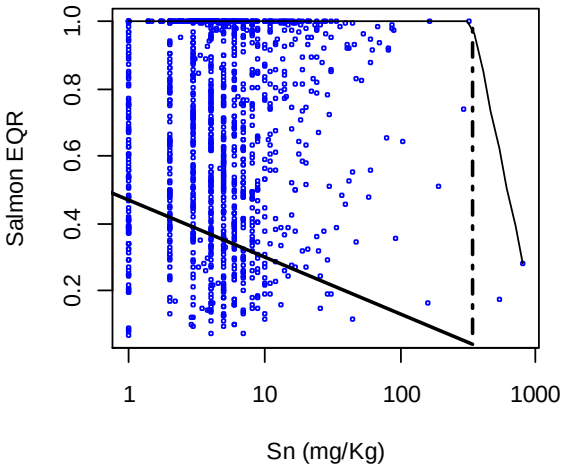
1
10
100
1000
Sn (mg/Kg)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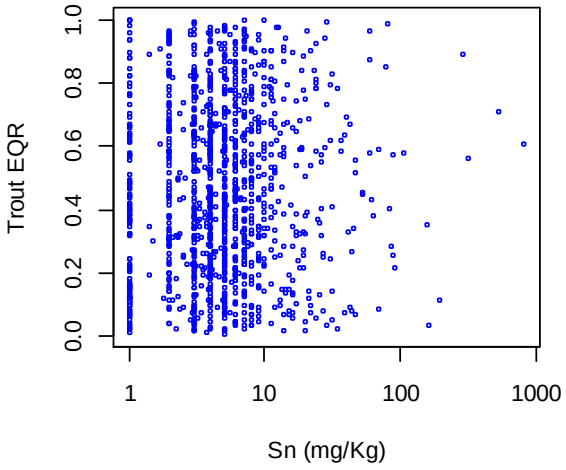
0.4
0.6
0.8
1.0
1
10
100
1000
Sn (mg/Kg)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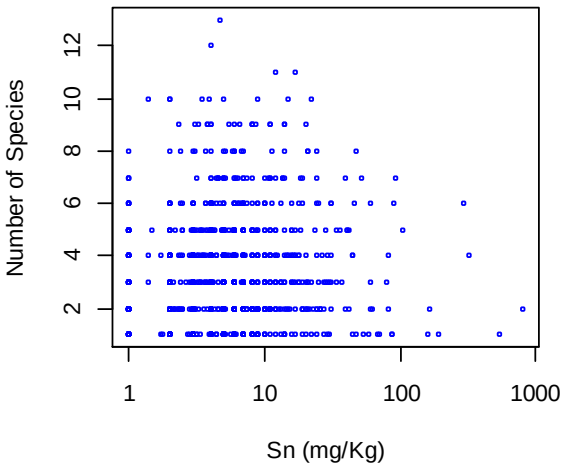
0.2
0.4
0.6
0.8
1.0
1
10
100
1000
Sn (mg/Kg)


Number of Species
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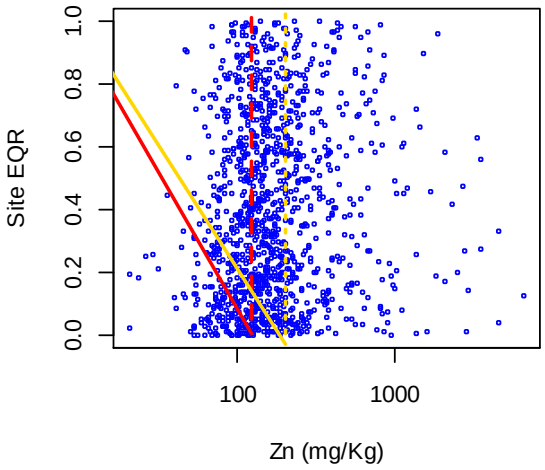
4
6
8
10
12
1
10
100
1000
Sn (mg/Kg)

Zinc


Site EQR
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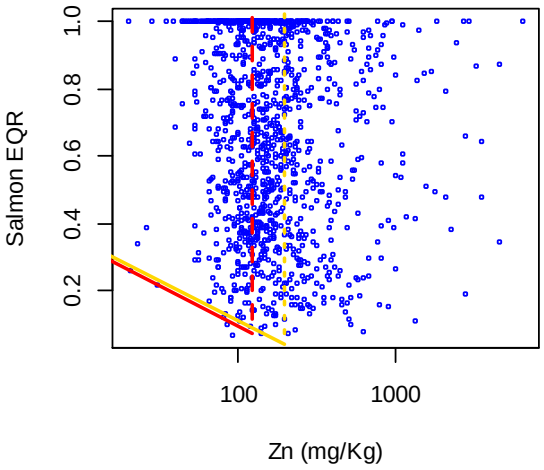
0.2
0.4
0.6
0.8
1.0
100
1000
Zn (mg/Kg)


Salmon EQR
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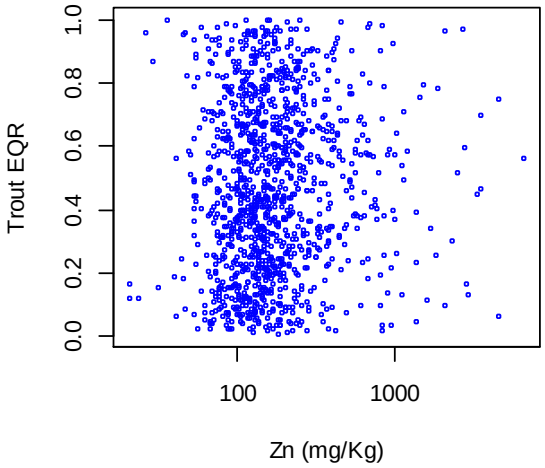
0.4
0.6
0.8
1.0
100
1000
Zn (mg/Kg)


Trout EQR
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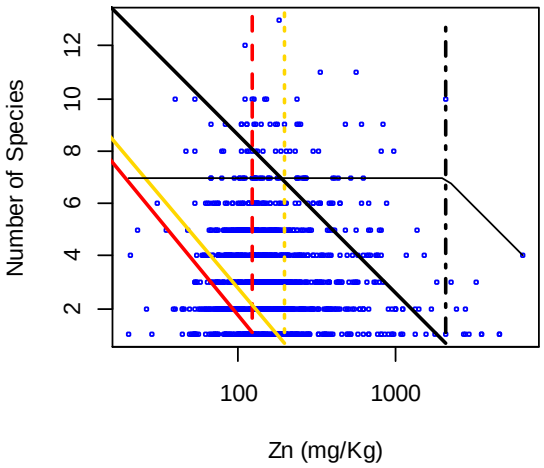
0.2
0.4
0.6
0.8
1.0
100
1000
Zn (mg/Kg)


Number of Species
2
4
6
8
10
12
100
1000
Zn (mg/Kg)

Appendix 3 Key to abbreviated taxon names

Abbreviated Taxon Name	Full Name
Polyfeli	*Polycelis felina*
Phagvitt	*Phagocata vitta*
Crenalpi	*Crenobia alpina*
Nematomo	Nematomorpha
Nematoda	Nematoda
Potaanti	*Potamopyrgus antipodarum*
Physidae	Physidae
Lymnaeid	Lymnaeidae
Ancyfluv	*Ancylus fluviatilis*
Pisidium	*Pisidium* sp.
Lumbricu	Lumbriculidae
Enchytra	Enchytraeidae
Naididae	Naididae
Tubifici	Tubificidae
Lumbrici	Lumbricidae
Erpoocto	*Erpobdella octoculata*
Hydracar	Hydracarina
Aselaqua	*Asellus aquaticus*
Cranpseu	*Crangonyx pseudogracilis*
Gammpule	*Gammarus pulex*
Baetrhod	*Baetis rhodani*
Baetvern	*Baetis vernus*
Baetscgp	*Baetis scambus* group
Centlute	*Centroptilum luteolum*
Alaimuti	*Alainites muticus*
Nigrnige	*Nigrobaetis niger*
Rhithrog	*Rhithrogena* sp.
Ecdyonur	*Ecdyonurus* sp.
Eleclate	*Electrogena lateralis*
Parasubm	*Paraleptophlebia submarginata*
Ephedani	*Ephemera danica*
Serrigni	*Serratella ignita*
Caenrivu	*Caenis rivulorum*
Bracrisi	*Brachyptera risi*
Protmeye	*Protonemura meyeri*
Protprae	*Protonemura praecox*
Amphsulc	*Amphinemura sulcicollis*
Nemupict	*Nemurella picteti*
Nemoavic	*Nemoura avicularis*
Nemocagp	*Nemoura cambrica* group
Leucfusc	*Leuctra fusca*
Leuchipp	*Leuctra hippopus*
Leuciner	*Leuctra inermis*
Leucnigr	*Leuctra nigra*
Perlmicr	*Perlodes microcephalus*
Isopgram	*Isoperla grammatica*
Perlidae	Perlidae
Chlorope	Chloroperlidae
Calopter	*Calopteryx* sp.
Cordbolt	*Cordulegaster boltonii*
Velicap	*Velia (Plesiovelia) caprai*
Oreosanm	*Oreodytes sanmarkii*
Oreosept	*Oreodytes septentrionalis*
Platmacu	*Platambus maculatus*
Agabus	*Agabus* sp.
Orecvill	*Orectochilus villosus*
Hydrgrac	*Hydraena gracilis*
Elodes	*Elodes* sp.
Elmiaena	*Elmis aenea*
Esolpara	*Esolus parallelepipedus*
Limnvolc	*Limnius volckmari*
Oulitube	*Oulimnius tuberculatus*
Rhyacoph	*Rhyacophila* sp.
Glossoso	*Glossosoma* sp.
Agapetus	*Agapetus* sp.
Hydropti	*Hydroptila* sp.
Ithytric	*Ithytrichia* sp.
Philmont	*Philopotamus montanus*
Wormaldi	*Wormaldia* sp.
Lype sp.	*Lype* sp.
Plectroc	*Plectrocnemia* sp.
Polycent	*Polycentropus* sp.
Hydrinst	*Hydropsyche instabilis*
Hydrpell	*Hydropsyche pellucidula*
Hydrsilt	*Hydropsyche siltalai*
Diplfeli	*Diplectrona felix*
Lepidost	Lepidostomatidae
Limnephi	Limnephilidae
Halesus	*Halesus* sp.
Potagrp	*Potamophylax* group
Drusinae	Drusinae
Silopall	*Silo pallipes*
Seripers	*Sericostoma personatum*
Odonalbi	*Odontocerum albicorne*
Athripso	*Athripsodes* sp.
Mystacid	*Mystacides* sp.
Adicella	*Adicella reducta*
Oecetis	*Oecetis* sp.
Tipumngp	*Tipula (Yamatotipula) montium* group
Tipumaxi	*Tipula (Acutipula) maxima*
Eloeophi	*Eloeophila* sp.
Pedicia	*Pedicia* sp.
Dicranot	*Dicranota* sp.
Pericoma	*Pericoma* group
Dixapube	*Dixa puberula*
Ceratopo	Ceratopogonidae
Proshirt	*Prosimulium hirtipes*
Simucvgp	*Simulium (Nevermannia) cryophilum-vernum* group
Simuaugp	*Simulium (Eusimulium) aureum* group
Simurept	*Simulium (Simulium) reptans*
Simuargp	*Simulium (Simulium) argyreatum* group
Simuorgp	*Simulium (Simulium) ornatum* group
Tanypodi	Tanypodinae [sub-family]
Diamesin	Diamesinae [sub-family]
Orthocla	Orthocladiinae [sub-family]
Chironom	Chironomini [tribe]
Tanytars	Tanytarsini [tribe]
Atheibis	*Atherix ibis*
Ibismarg	*Ibisia marginata*
Clinocer	Clinocerinae
Hemerodr	Hemerodrominae
Limnripa	*Limnophora riparia*

Appendix 4 Key to abbreviated variable names

Abbreviated Variable Name	Variable
logDfS	log Distance from source (km)
logAlt	log Altitude (m asl)
logSlp	log Slope (mkm^{-1})
StrOrd	Strahler stream order
logWid	log Stream width (m)
logDep	log Stream Depth (cm)
VeloCat	Velocity category
Subsphi	Substratum phi score
Conduct	Conductivity ($\mu S\ cm^{-1}$)
lgCdBaet	log (x+1) Cd in Baetidae ($\mu g\ g^{-1}$)
lgCuBaet	log (x+1) Cu in Baetidae ($\mu g\ g^{-1}$)
lgNiBaet	log (x+1) Ni in Baetidae ($\mu g\ g^{-1}$)
lgPbBaet	log (x+1) Pb in Baetidae ($\mu g\ g^{-1}$)
lgZnBaet	log (x+1) Zn in Baetidae ($\mu g\ g^{-1}$)
lgCuHydro	log (x+1) Cu in Hydropsychidae ($\mu g\ g^{-1}$)
lgNiHydro	log (x+1) Ni in Hydropsychidae ($\mu g\ g^{-1}$)
lgPbHydro	log (x+1) Pb in Hydropsychidae ($\mu g\ g^{-1}$)
lgZnHydro	log (x+1) Zn in Hydropsychidae ($\mu g\ g^{-1}$)

Appendix 5 Regression equations used to model missing *Baetis* tissue metal concentrations

Catchment	Site	As $\mu g\ g^{-1}$	Cd $\mu g\ g^{-1}$	Cu $\mu g\ g^{-1}$	Ni $\mu g\ g^{-1}$	Pb $\mu g\ g^{-1}$	Zn $\mu g\ g^{-1}$
A	ds	1.039 + 0.5861 log10(As_Hydrop)	0.9628 + 0.4086 log10(Cd_Hydrop)	0.9697 + 0.6385 log10(Cu_Hydrop)	0.7590 + 0.3095 log10(Ni_Hydrop)	0.0235 + 0.9355 log10(Pb_Hydrop)	0.8039 + 0.9675 log10(Zn_Hydrop)
I	ds	1.039 + 0.5861 log10(As_Hydrop)	0.9628 + 0.4086 log10(Cd_Hydrop)	0.9697 + 0.6385 log10(Cu_Hydrop)	0.7590 + 0.3095 log10(Ni_Hydrop)	0.1016 + 0.7545 log10(Pb_Hydrop)	- 0.230 + 1.288 log10(Zn_Hydrop)
I	Ind Cont	1.039 + 0.5861 log10(As_Hydrop)	0.9628 + 0.4086 log10(Cd_Hydrop)	0.9697 + 0.6385 log10(Cu_Hydrop)	0.7590 + 0.3095 log10(Ni_Hydrop)	0.1016 + 0.7545 log10(Pb_Hydrop)	- 0.230 + 1.288 log10(Zn_Hydrop)
I	us	1.039 + 0.5861 log10(As_Hydrop)	0.9628 + 0.4086 log10(Cd_Hydrop)	0.9697 + 0.6385 log10(Cu_Hydrop)	0.7590 + 0.3095 log10(Ni_Hydrop)	0.1016 + 0.7545 log10(Pb_Hydrop)	- 0.230 + 1.288 log10(Zn_Hydrop)
J	ds depo	1.039 + 0.5861 log10(As_Hydrop)	0.9628 + 0.4086 log10(Cd_Hydrop)	0.9697 + 0.6385 log10(Cu_Hydrop)	0.7590 + 0.3095 log10(Ni_Hydrop)	0.1016 + 0.7545 log10(Pb_Hydrop)	- 0.230 + 1.288 log10(Zn_Hydrop)
J	ds eros	1.039 + 0.5861 log10(As_Hydrop)	0.9628 + 0.4086 log10(Cd_Hydrop)	0.9697 + 0.6385 log10(Cu_Hydrop)	0.7590 + 0.3095 log10(Ni_Hydrop)	0.1016 + 0.7545 log10(Pb_Hydrop)	- 0.230 + 1.288 log10(Zn_Hydrop)
J	Ind Cont	1.039 + 0.5861 log10(As_Hydrop)	0.9628 + 0.4086 log10(Cd_Hydrop)	0.9697 + 0.6385 log10(Cu_Hydrop)	0.7590 + 0.3095 log10(Ni_Hydrop)	0.1016 + 0.7545 log10(Pb_Hydrop)	- 0.230 + 1.288 log10(Zn_Hydrop)
K	ds	*- 0.1578 + 0.2980 log10(As_Leuc)*	0.0212 + 0.6450 log10(Cd_Leuc)	0.9565 + 0.3153 log10(Cu_Leuc)	0.6072 + 0.3218 log10(Ni_Leuc)	0.2680 + 0.5564 log10(Pb_Leuc)	- 2.197 + 3.594 log10(Zn_Leuc) - 0.6409 log10(Zn_Leuc)^2
K	ds eros	*- 0.1578 + 0.2980 log10(As_Leuc)*	0.0212 + 0.6450 log10(Cd_Leuc)	0.9565 + 0.3153 log10(Cu_Leuc)	0.6072 + 0.3218 log10(Ni_Leuc)	0.2680 + 0.5564 log10(Pb_Leuc)	- 2.197 + 3.594 log10(Zn_Leuc) - 0.6409 log10(Zn_Leuc)^2
K	Ind Cont	*- 0.1578 + 0.2980 log10(As_Leuc)*	0.0212 + 0.6450 log10(Cd_Leuc)	0.9565 + 0.3153 log10(Cu_Leuc)	0.6072 + 0.3218 log10(Ni_Leuc)	0.2680 + 0.5564 log10(Pb_Leuc)	- 2.197 + 3.594 log10(Zn_Leuc) - 0.6409 log10(Zn_Leuc)^2
K	us	*- 0.1578 + 0.2980 log10(As_Leuc)*	0.0212 + 0.6450 log10(Cd_Leuc)	0.9565 + 0.3153 log10(Cu_Leuc)	0.6072 + 0.3218 log10(Ni_Leuc)	0.2680 + 0.5564 log10(Pb_Leuc)	- 2.197 + 3.594 log10(Zn_Leuc) - 0.6409 log10(Zn_Leuc)^2
Q	us	*- 0.1578 + 0.2980 log10(As_Leuc)*	0.0212 + 0.6450 log10(Cd_Leuc)	1.885 - 0.5275 log10(Cu_Rhya)	0.6072 + 0.3218 log10(Ni_Leuc)	- 0.0413 + 1.066 log10(Pb_Rhya)	- 1.291 + 1.680 log10(Zn_Rhya)
T	us	*- 0.1578 + 0.2980 log10(As_Leuc)*	0.0212 + 0.6450 log10(Cd_Leuc)	1.885 - 0.5275 log10(Cu_Rhya)	0.6072 + 0.3218 log10(Ni_Leuc)	- 0.0413 + 1.066 log10(Pb_Rhya)	- 1.291 + 1.680 log10(Zn_Rhya)

Appendix 6 Benthic macroinvertebrate taxa recorded across the 99 stream sites.

TAXON_NAME	No. of occurrences
Microturbellaria	1
Polycelis felina	39
Polycelis nigra group	3
Phagocata vitta	19
Crenobia alpina	27
Dendrocoelum lacteum	3
Nemertea	3
Nematomorpha	5
Nematoda	10
Trichocercidae	1
Potamopyrgus antipodarum	22
Physidae	5
Lymnaeidae	9
Planorbidae	2
Ancylus fluviatilis	23
Pisidium sp.	41
Lumbriculidae	60
Haplotaxidae	1
Enchytraeidae	36
Naididae	46
Tubificidae	44
Lumbricidae	53
Piscicola geometra	2
Glossiphonia complanata	3
Helobdella stagnalis	2
Erpobdella octoculata	6
Trocheta sp.	1
Hydracarina	52
Oribatei	4
Cladocera	1
Ostracoda	3
Asellus aquaticus	10
Proasellus meridianus	2
Orchestia cavimana	2
Crangonyx pseudogracilis	6
Gammarus pulex	40
Collembola	1
Siphlonurus sp.	2
Baetis rhodani	92
Baetis vernus	9
Baetis scambus group	6
Centroptilum luteolum	11
Alainites muticus	55
Nigrobaetis niger	6
Rhithrogena sp.	79
Heptagenia sulphurea	3
Ecdyonurus sp.	57
Electrogena lateralis	48
Leptophlebia marginata	5
Paraleptophlebia sp.	24
Habrophlebia fusca	1
Ephemera danica	10
Serratella ignita	14
Caenis rivulorum	14
Taeniopteryx nebulosa	2
Brachyptera risi	45
Protonemura meyeri	67
Protonemura montana	1
Protonemura praecox	7
Amphinemura standfussi	2
Amphinemura sulcicollis	66
Nemurella picteti	6
Nemoura avicularis	17
Nemoura cinerea	1
Nemoura cambrica group	16
Leuctra fusca	17
Leuctra geniculata	4
Leuctra hippopus	44
Leuctra inermis	76
Leuctra nigra	22
Perlodes microcephalus	44
Diura bicaudata	3
Isoperla grammatica	80
Dinocras cephalotes	16
Perla bipunctata	21
Chloroperla tripunctata	36
Siphonoperla torrentium	75
Pyrrhosoma nymphula	1
Calopteryx sp.	8
Cordulegaster boltonii	14
Hydrometra stagnorum	1
Velia (Plesiovelia) caprai	7
Aquarius najas	3
Micronecta sp.	1
Agriotypus armatus	1
Brychius elevatus	1
Oreodytes davisii	6
Oreodytes sanmarkii	46
Oreodytes septentrionalis	9
Platambus maculatus	11
Agabus sp.	5
Gyrinus natator group	1
Gyrinus urinator	1
Orectochilus villosus	31
Helophorus (Rhopalohelophorus) brevipalpis	2
Anacaena globulus	3
Cercyon sp.	1
Ochthebius exsculptus	1
Hydraena sp.	63
Limnebius truncatellus	2
Elodes sp.	19
Cyphon sp.	1
Hydrocyphon sp.	7
Scirtes sp.	1
Dryops sp.	3
Elmis aenea	84
Esolus parallelepipedus	49
Limnius volckmari	78
Oulimnius tuberculatus	33

Sialis fuliginosa	3
Sialis lutaria	1
Osmylus fulvicephalus	1
Rhyacophila dorsalis	91
Rhyacophila munda	3
Glossosoma sp.	16
Agapetus sp.	23
Hydroptila sp.	9
Oxyethira sp.	1
Ithytrichia sp.	15
Philopotamus montanus	17
Wormaldia sp.	10
Lype sp.	6
Psychomyia pusilla	2
Tinodes waeneri	1
Plectrocnemia sp.	30
Polycentropus sp.	37
Hydropsyche angustipennis	1
Hydropsyche fulvipes	1
Hydropsyche instabilis	34
Hydropsyche pellucidula	13
Hydropsyche siltalai	78
Diplectrona felix	11
Lepidostomatidae	46
Limnephilidae	69
Allogamus auricollis	4
Halesus sp.	45
Melampophylax mucoreus	1
Micropterna lateralis	1
Micropterna sequax	1
Potamophylax sp.	50
Chaetopteryx villosa	7
Limnephilus lunatus	1
Drusinae	31
Silo nigricornis	3
Silo pallipes	51
Beraea maurus	2
Sericostoma personatum	61
Odontocerum albicorne	44
Athripsodes sp.	6
Mystacides sp.	10
Adicella reducta	8
Oecetis sp.	9
Tipula (Yamatotipula) montium group	11
Tipula (Acutipula) maxima group	9
Antocha (Antocha) vitripennis	1
Limnophila sp.	1
Eloeophila sp.	26
Pilaria sp.	1
Hexatoma sp.	2
Lipsothrix sp.	1
Molophilus sp.	2
Pedicia sp.	11
Dicranota sp.	76
Tricyphona sp.	2
Psychoda group	2
Pericoma group	26

Dixa dilatata	1
Dixa nebulosa	1
Dixa puberula	8
Dixa maculata complex	1
Ceratopogonidae	23
Prosimulium sp.	18
Simulium (Nevermannia) costatum	1
Simulium (Nevermannia) cryophilum-vernum group	64
Simulium (Nevermannia) angustitarse group	4
Simulium (Eusimulium) aureum group	11
Simulium (Wilhelmia) sp.	1
Simulium (Simulium) reptans	6
Simulium (Simulium) argyreatum group	67
Simulium (Simulium) ornatum group	36
Tanypodinae [sub-family]	48
Diamesinae [sub-family]	30
Prodiamesinae [sub-family]	3
Orthocladiinae [sub-family]	97
Chironomini [tribe]	43
Tanytarsini [tribe]	57
Rhagionidae	1
Chrysops sp.	1
Tabanus sp.	1
Atherix ibis	5
Ibisia marginata	16
Clinocerinae	58
Hemerodrominae	48
Ephydridae	2
Scathophagidae	1
Limnophora riparia	8

Appendix 7 Source apportionment statistical results

Table A7.1: The results of the Lilliefors test for Normality using the source and sediment tracer data measured for the Afon Ystwyth study catchment.

Property	P-value	Property	P-value	Property	P-value
Al	0.500	Sc	0.500	Nd	0.001*
Ba	0.028*	Co	0.003*	Sm	0.001*
Cr	0.154	Ga	0.001*	Eu	0.001*
Cu	0.001*	Ge	0.001*	Gd	0.001*
Fe	0.500	As	0.001*	Tb	0.006*
K	0.490	Rb	0.001*	Dy	0.001*
Li	0.123	Y	0.002*	Ho	0.001*
Mg	0.500	Zr	0.001*	Er	0.001*
Mn	0.001*	Mo	0.001*	Tm	0.001*
Na	0.284	Cd	0.001*	Yb	0.001*
Ni	0.292	Sn	0.001*	Lu	0.001*
Pb	0.001*	Sb	0.001*	Hf	0.001*
Sr	0.001*	Cs	0.001*	Tl	0.001*
Ti	0.001*	La	0.001*	Bi	0.320
V	0.041*	Ce	0.001*	U	0.197
Zn	0.001*	Pr	0.001*		

* statistically significant values at $p \leq 0.05$

Table A7.2: The results of the Lilliefors test for Normality using the source and sediment tracer data measured for the Arkle Beck study catchment.

Property	P-value	Property	P-value	Property	P-value
Al	0.037*	Sc	0.435	Nd	0.001*
Ba	0.297	Co	0.001*	Sm	0.001*
Cr	0.001*	Ga	0.107	Eu	0.500
Cu	0.001*	Ge	0.019*	Gd	0.001*
Fe	0.121	As	0.001*	Tb	0.001*
K	0.001*	Rb	0.001*	Dy	0.022*
Li	0.001*	Y	0.053	Ho	0.098
Mg	0.001*	Zr	0.001*	Er	0.144
Mn	0.001*	Mo	0.001*	Tm	0.002*
Na	0.001*	Cd	0.001*	Yb	0.225
Ni	0.032*	Sn	0.001*	Lu	0.001*
Pb	0.001*	Sb	0.001*	Hf	0.001*
Sr	0.001*	Cs	0.001*	Tl	0.001*
Ti	0.001*	La	0.001*	Bi	0.006*
V	0.001*	Ce	0.001*	U	0.002*
Zn	0.001*	Pr	0.001*		

* statistically significant values at $p \leq 0.05$

Table A7.3: The results of the Lilliefors test for Normality using the source and sediment tracer data measured for the Bedburn Beck study catchment.

Property	P-value	Property	P-value	Property	P-value
Al	0.084	Sc	0.500	Nd	0.453
Ba	0.007*	Co	0.109	Sm	0.285
Cr	0.026*	Ga	0.064	Eu	0.001*
Cu	0.014*	Ge	0.346	Gd	0.001*
Fe	0.500	As	0.002*	Tb	0.009*
K	0.001*	Rb	0.001*	Dy	0.001*
Li	0.004*	Y	0.001*	Ho	0.001*
Mg	0.001*	Zr	0.001*	Er	0.001*
Mn	0.052	Mo	0.001*	Tm	0.001*
Na	0.002*	Cd	0.001*	Yb	0.078
Ni	0.058	Sn	0.101	Lu	0.001*
Pb	0.001*	Sb	0.001*	Hf	0.001*
Sr	0.001*	Cs	0.002*	Tl	0.113
Ti	0.069	La	0.500	Bi	0.001*
V	0.001*	Ce	0.500	U	0.059
Zn	0.001*	Pr	0.500		

* statistically significant values at $p \leq 0.05$

Table A7.4: The results of the Lilliefors test for Normality using the source and sediment tracer data measured for the Bolingey Stream study catchment.

Property	P-value	Property	P-value	Property	P-value
Al	0.081	Zn	0.001*	Pr	0.500
Ba	0.067	Sc	0.314	Nd	0.162
Cr	0.411	Co	0.001*	Sm	0.014*
Cu	0.001*	Ga	0.376	Eu	0.001*
Fe	0.001*	Ge	0.500	Gd	0.202
K	0.259	As	0.001*	Tb	0.500
Li	0.144	Rb	0.487	Dy	0.385
Mg	0.500	Y	0.001*	Ho	0.001*
Mn	0.006*	Zr	0.001*	Er	0.001*
Na	0.017*	Cd	0.001*	Tm	0.001*
Ni	0.500	Sn	0.001*	Yb	0.001*
Pb	0.001*	Sb	0.001*	Hf	0.001*
Sr	0.005*	Cs	0.015*	Tl	0.003*
Ti	0.001*	La	0.218	Bi	0.001*
V	0.001*	Ce	0.277	U	0.001*

* statistically significant values at $p \leq 0.05$

Table A7.5: The results of the Lilliefors test for Normality using the source and sediment tracer data measured for the Egglestone Beck study catchment.

Property	P-value	Property	P-value	Property	P-value
Al	0.169	Sc	0.266	Nd	0.500
Ba	0.002*	Co	0.192	Sm	0.282
Cr	0.094	Ga	0.300	Eu	0.007*
Cu	0.001*	Ge	0.301	Gd	0.498
Fe	0.500	As	0.049*	Tb	0.091
K	0.046*	Rb	0.030*	Dy	0.018*
Li	0.340	Y	0.003*	Ho	0.030*
Mg	0.216	Zr	0.001*	Er	0.164
Mn	0.105	Mo	0.001*	Tm	0.001*
Na	0.115	Cd	0.045*	Yb	0.364
Ni	0.500	Sn	0.001*	Lu	0.001*
Pb	0.053	Sb	0.001*	Hf	0.001*
Sr	0.001*	Cs	0.338	Tl	0.001*
Ti	0.001*	La	0.083	Bi	0.001*
V	0.424	Ce	0.111	U	0.001*
Zn	0.023*	Pr	0.066		

* statistically significant values at $p \leq 0.05$

Table A7.6: The results of the Lilliefors test for Normality using the source and sediment tracer data measured for the Hayle study catchment.

Property	P-value	Property	P-value	Property	P-value
Al	0.500	Sc	0.001*	Nd	0.001*
Ba	0.500	Co	0.001*	Sm	0.001*
Cr	0.002*	Ga	0.001*	Eu	0.001*
Cu	0.001*	Ge	0.003*	Gd	0.001*
Fe	0.001*	As	0.001*	Tb	0.001*
K	0.001*	Rb	0.001*	Dy	0.001*
Li	0.047*	Y	0.001*	Ho	0.001*
Mg	0.500	Zr	0.001*	Er	0.001*
Mn	0.002*	Mo	0.001*	Tm	0.004*
Na	0.366	Cd	0.001*	Yb	0.001*
Ni	0.009*	Sn	0.002*	Lu	0.004*
Pb	0.001*	Sb	0.001*	Hf	0.001*
Sr	0.148	Cs	0.047*	Tl	0.003*
Ti	0.001*	La	0.001*	Bi	0.001*
V	0.009*	Ce	0.001*	U	0.001*
Zn	0.001*	Pr	0.001*		

* statistically significant values at $p \leq 0.05$

Table A7.7: The results of the Lilliefors test for Normality using the source and sediment tracer data measured for the Hudeshope Beck study catchment.

Property	P-value	Property	P-value	Property	P-value
Al	0.468	Zn	0.001*	Ce	0.104
Ba	0.346	Sc	0.500	Pr	0.500
Cr	0.239	Co	0.008*	Nd	0.309
Cu	0.001*	Ga	0.120	Sm	0.500
Fe	0.186	Ge	0.079	Eu	0.001*
K	0.011*	As	0.001*	Gd	0.003*
Li	0.374	Rb	0.500	Tb	0.001*
Mg	0.001*	Y	0.001*	Dy	0.001*
Mn	0.113	Zr	0.001*	Ho	0.001*
Na	0.001*	Mo	0.001*	Er	0.008*
Ni	0.169	Cd	0.001*	Tm	0.001*
Pb	0.001*	Sn	0.004*	Yb	0.008*
Sr	0.001*	Sb	0.001*	Lu	0.001*
Ti	0.001*	Cs	0.461	Tl	0.445
V	0.001*	La	0.500	U	0.050

* statistically significant values at $p \leq 0.05$

Table A7.8: The results of the Lilliefors test for Normality using the source and sediment tracer data measured for the Mardle study catchment.

Property	P-value	Property	P-value	Property	P-value
Al	0.008*	Sc	0.002*	Nd	0.500
Ba	0.001*	Co	0.001*	Sm	0.399
Cr	0.040*	Ga	0.041*	Eu	0.500
Cu	0.001*	Ge	0.243	Gd	0.500
Fe	0.001*	As	0.001*	Tb	0.064
K	0.001*	Rb	0.002*	Dy	0.099
Li	0.364	Y	0.084	Ho	0.046*
Mg	0.335	Zr	0.001*	Er	0.385
Mn	0.001*	Mo	0.001*	Tm	0.473
Na	0.001*	Cd	0.001*	Yb	0.176
Ni	0.024*	Sn	0.001*	Lu	0.215
Pb	0.001*	Sb	0.001*	Hf	0.001*
Sr	0.001*	Cs	0.500	Tl	0.012*
Ti	0.001*	La	0.416	Bi	0.001*
V	0.037*	Ce	0.204	U	0.026*
Zn	0.001*	Pr	0.500		

* statistically significant values at $p \leq 0.05$

Table A7.9: The results of the Lilliefors test for Normality using the source and sediment tracer data measured for the Nant Magwr study catchment.

Property	P-value	Property	P-value	Property	P-value
Al	0.279	Sc	0.032*	Nd	0.001*
Ba	0.101	Co	0.500	Sm	0.001*
Cr	0.001*	Ga	0.001*	Eu	0.001*
Cu	0.001*	Ge	0.001*	Gd	0.001*
Fe	0.054	As	0.001*	Tb	0.001*
K	0.500	Rb	0.190	Dy	0.001*
Li	0.026*	Y	0.009*	Ho	0.002*
Mg	0.500	Zr	0.001*	Er	0.004*
Mn	0.001*	Mo	0.001*	Tm	0.008*
Na	0.026*	Cd	0.001*	Yb	0.147
Ni	0.003*	Sn	0.001*	Lu	0.058
Pb	0.001*	Sb	0.001*	Hf	0.001*
Sr	0.001*	Cs	0.007*	Tl	0.001*
Ti	0.001*	La	0.001*	Bi	0.344
V	0.460	Ce	0.001*	U	0.005*
Zn	0.001*	Pr	0.001*		

* statistically significant values at $p \leq 0.05$

Table A7.10: The results of the Lilliefors test for Normality using the source and sediment tracer data measured for the Porthleven Stream study catchment.

Property	P-value	Property	P-value	Property	P-value
Al	0.054	Sc	0.097	Sm	0.009*
Ba	0.500	Co	0.001*	Eu	0.001*
Cr	0.168	Ga	0.004*	Gd	0.005*
Cu	0.001*	Ge	0.001*	Tb	0.442
Fe	0.001*	As	0.001*	Dy	0.293
K	0.004*	Rb	0.500	Ho	0.037*
Li	0.138	Y	0.116	Er	0.088
Mg	0.001*	Zr	0.001*	Tm	0.361
Mn	0.001*	Cd	0.001*	Yb	0.177
Na	0.066	Sn	0.001*	Lu	0.086
Ni	0.008*	Sb	0.001*	Hf	0.001*
Pb	0.001*	Cs	0.500	Tl	0.001*
Sr	0.003*	La	0.001*	Bi	0.001*
Ti	0.314	Ce	0.014*	U	0.027*
V	0.035*	Pr	0.001*		
Zn	0.007*	Nd	0.004*		

* statistically significant values at $p \leq 0.05$

Table A7.11: The results of the Lilliefors test for Normality using the source and sediment tracer data measured for the Rea Brook study catchment.

Property	P-value	Property	P-value	Property	P-value
Al	0.341	Sc	0.001*	Nd	0.001*
Ba	0.001*	Co	0.001*	Sm	0.001*
Cr	0.131	Ga	0.287	Eu	0.001*
Cu	0.001*	Ge	0.006*	Gd	0.001*
Fe	0.165	As	0.001*	Tb	0.001*
K	0.423	Rb	0.247	Dy	0.001*
Li	0.069	Y	0.001*	Ho	0.001*
Mg	0.001*	Zr	0.001*	Er	0.001*
Mn	0.001*	Mo	0.001*	Tm	0.001*
Na	0.001*	Cd	0.001*	Yb	0.021*
Ni	0.001*	Sn	0.001*	Lu	0.006*
Pb	0.001*	Sb	0.001*	Hf	0.001*
Sr	0.001*	Cs	0.001*	Tl	0.001*
Ti	0.001*	La	0.001*	Bi	0.001*
V	0.001*	Ce	0.001*	U	0.010*
Zn	0.001*	Pr	0.001*		

* statistically significant values at $p \leq 0.05$

Table A7.12: The results of the Lilliefors test for Normality using the source and sediment tracer data measured for the Red Tarn Beck study catchment.

Property	P-value	Property	P-value	Property	P-value
Al	0.162	Sc	0.039*	Nd	0.029*
Ba	0.002*	Co	0.001*	Sm	0.331
Cr	0.001*	Ga	0.126	Eu	0.001*
Cu	0.001*	Ge	0.163	Gd	0.079
Fe	0.160	As	0.001*	Tb	0.001*
K	0.030*	Rb	0.001*	Dy	0.500
Li	0.181	Y	0.304	Ho	0.061
Mg	0.085	Zr	0.020*	Er	0.209
Mn	0.001*	Mo	0.001*	Tm	0.004*
Na	0.005*	Cd	0.001*	Yb	0.175
Ni	0.180	Sn	0.041*	Lu	0.001*
Pb	0.001*	Sb	0.001*	Hf	0.001*
Sr	0.001*	Cs	0.001*	Bi	0.016*
Ti	0.001*	La	0.021*	U	0.001*
V	0.001*	Ce	0.040*		
Zn	0.001*	Pr	0.086		

* statistically significant values at $p \leq 0.05$

Table A7.13: The results of the Lilliefors test for Normality using the source and sediment tracer data measured for the River Burn (Tavy) study catchment.

Property	P-value	Property	P-value	Property	P-value
Al	0.340	Zn	0.001*	Pr	0.030*
Ba	0.001*	Sc	0.017*	Nd	0.006*
Cr	0.079	Co	0.001*	Sm	0.003*
Cu	0.001*	Ga	0.500	Eu	0.001*
Fe	0.001*	Ge	0.001*	Gd	0.001*
K	0.001*	As	0.001*	Tb	0.208
Li	0.124	Rb	0.041*	Dy	0.001*
Mg	0.001*	Y	0.001*	Er	0.001*
Mn	0.001*	Mo	0.001*	Tm	0.001*
Na	0.001*	Cd	0.001*	Yb	0.001*
Ni	0.001*	Sn	0.001*	Lu	0.001*
Pb	0.001*	Sb	0.500	Hf	0.001*
Sr	0.001*	Cs	0.081	Tl	0.001*
Ti	0.006*	La	0.106	Bi	0.001*
V	0.001*	Ce	0.007*	U	0.121

* statistically significant values at $p \leq 0.05$

Table A7.14: The results of the Lilliefors test for Normality using the source and sediment tracer data measured for the River East Allen study catchment.

Property	P-value	Property	P-value	Property	P-value
Al	0.001*	Sc	0.500	Nd	0.056
Ba	0.039*	Co	0.001*	Sm	0.457
Cr	0.385	Ga	0.003*	Eu	0.001*
Cu	0.001*	Ge	0.004*	Gd	0.500
Fe	0.236	As	0.197	Tb	0.262
K	0.005*	Rb	0.500	Dy	0.148
Li	0.026*	Y	0.021*	Ho	0.262
Mg	0.001*	Zr	0.001*	Er	0.336
Mn	0.001*	Mo	0.001*	Tm	0.364
Na	0.001*	Cd	0.001*	Yb	0.228
Ni	0.158	Sn	0.001*	Lu	0.244
Pb	0.001*	Sb	0.001*	Hf	0.001*
Sr	0.001*	Cs	0.052	Tl	0.254
Ti	0.001*	La	0.094	Bi	0.002*
V	0.003*	Ce	0.068	U	0.319
Zn	0.001*	Pr	0.157		

* statistically significant values at $p \leq 0.05$

Table A7.15: The results of the Lilliefors test for Normality using the source and sediment tracer data measured for the River Greta study catchment.

Property	P-value	Property	P-value	Property	P-value
Al	0.047*	Sc	0.500	Nd	0.014*
Ba	0.002*	Co	0.001*	Sm	0.117
Cr	0.001*	Ga	0.270	Eu	0.500
Cu	0.090	Ge	0.099	Gd	0.299
Fe	0.047*	As	0.001*	Tb	0.001*
K	0.179	Rb	0.007*	Dy	0.500
Li	0.408	Y	0.119	Ho	0.001*
Mg	0.001*	Zr	0.001*	Er	0.001*
Mn	0.001*	Mo	0.001*	Tm	0.001*
Na	0.001*	Cd	0.028*	Yb	0.008*
Ni	0.236	Sn	0.001*	Lu	0.001*
Pb	0.001*	Sb	0.001*	Hf	0.001*
Sr	0.001*	Cs	0.001*	Tl	0.001*
Ti	0.001*	La	0.014*	Bi	0.001*
V	0.001*	Ce	0.022*	U	0.001*
Zn	0.007*	Pr	0.015*		

* statistically significant values at $p \leq 0.05$

Table A7.16: The results of the Lilliefors test for Normality using the source and sediment tracer data measured for the River South Tyne study catchment.

Property	P-value	Property	P-value	Property	P-value
Al	0.157	Sc	0.500	Nd	0.004*
Ba	0.001*	Co	0.001*	Sm	0.193
Cr	0.001*	Ga	0.005*	Eu	0.001*
Cu	0.001*	Ge	0.068	Gd	0.016*
Fe	0.175	As	0.001*	Tb	0.055
K	0.012*	Rb	0.002*	Dy	0.266
Li	0.132	Y	0.001*	Ho	0.003*
Mg	0.001*	Zr	0.001*	Er	0.094
Mn	0.001*	Mo	0.001*	Tm	0.002*
Na	0.001*	Cd	0.001*	Yb	0.235
Ni	0.001*	Sn	0.001*	Lu	0.005*
Pb	0.001*	Sb	0.001*	Hf	0.001*
Sr	0.001*	Cs	0.001*	Tl	0.001*
Ti	0.001*	La	0.001*	Bi	0.500
V	0.014*	Ce	0.001*	U	0.004*
Zn	0.001*	Pr	0.001*		

* statistically significant values at $p \leq 0.05$

Table A7.17: The results of the Lilliefors test for Normality using the source and sediment tracer data measured for the St Lawrence Stream study catchment.

Property	P-value	Property	P-value	Property	P-value
Al	0.068	Sc	0.272	Nd	0.001*
Ba	0.001*	Co	0.001*	Sm	0.001*
Cr	0.073	Ga	0.194	Eu	0.001*
Cu	0.001*	Ge	0.001*	Gd	0.001*
Fe	0.001*	As	0.001*	Tb	0.001*
K	0.095	Rb	0.152	Dy	0.001*
Li	0.137	Y	0.001*	Ho	0.001*
Mg	0.001*	Zr	0.001*	Er	0.001*
Mn	0.001*	Mo	0.001*	Tm	0.001*
Na	0.001*	Cd	0.001*	Yb	0.001*
Ni	0.001*	Sn	0.001*	Lu	0.001*
Pb	0.264	Sb	0.001*	Hf	0.001*
Sr	0.001*	Cs	0.106	Tl	0.001*
Ti	0.016*	La	0.001*	Bi	0.001*
V	0.054	Ce	0.500	U	0.001*
Zn	0.001*	Pr	0.001*		

* statistically significant values at $p \leq 0.05$

Table A7.18: The results of the Lilliefors test for Normality using the source and sediment tracer data measured for the Wye study catchment.

Property	P-value	Property	P-value	Property	P-value
Al	0.021*	Sc	0.001*	Nd	0.001*
Ba	0.001*	Co	0.001*	Sm	0.036*
Cr	0.047*	Ga	0.001*	Eu	0.025*
Cu	0.286	Ge	0.001*	Gd	0.137
Fe	0.015*	As	0.001*	Tb	0.026*
K	0.184	Rb	0.308	Dy	0.014*
Li	0.399	Y	0.001*	Ho	0.027*
Mg	0.381	Zr	0.012*	Er	0.001*
Mn	0.001*	Mo	0.225	Tm	0.001*
Na	0.001*	Cd	0.001*	Yb	0.001*
Ni	0.001*	Sn	0.009*	Lu	0.001*
Pb	0.001*	Sb	0.001*	Hf	0.032*
Sr	0.001*	Cs	0.046*	Tl	0.001*
Ti	0.500	La	0.001*	Bi	0.051
V	0.001*	Ce	0.001*	U	0.050
Zn	0.001*	Pr	0.001*		

* statistically significant values at $p \leq 0.05$

Table A7.19: KW-H results for the Afon Ystwyth study catchment.

Property	H-value	p-value	Property	H-value	p-value
As	34.1	0.000	Sc	16.8	0.001
Cs	27.7	0.000	Sm	27.4	0.000
Cu	21.2	0.000	Sn	28.8	0.000
Ga	30.3	0.000	Tl	33.2	0.000
Hf	25.9	0.000	U	23.5	0.000
Pb	26.3	0.000	V	28.6	0.000
Sb	28.3	0.000	Zr	29.4	0.000

Table A7.20: KW-H results for the Arkle Beck study catchment.

Property	H-value	p-value	Property	H-value	p-value
As	8.5	0.015	Rb	13.1	0.001
Bi	23.3	0.000	Sb	12.8	0.002
Cs	12.8	0.002	Sc	12.1	0.002
Cu	13.3	0.001	Sn	23.0	0.000
Er	18.2	0.000	Tm	20.9	0.000
Eu	13.7	0.001	U	13.3	0.001
Ho	20.9	0.000	V	22.4	0.000
K	19.0	0.000	Yb	20.9	0.000
Li	5.6	0.060	Zn	11.5	0.003
Mo	18.6	0.000	Zr	20.9	0.000
Ni	16.6	0.000			

Table A7.21: KW-H results for the Bedburn Beck study catchment.

Property	H-value	p-value	Property	H-value	p-value
Al	19.8	0.000	Na	21.7	0.000
As	23.1	0.000	Pb	21.1	0.000
Cs	23.6	0.000	Rb	23.8	0.000
Cu	23.3	0.000	Sb	23.1	0.000
Dy	20.5	0.000	Sc	21.3	0.000
Er	21.8	0.000	Sr	14.0	0.003
Eu	20.5	0.000	Ti	17.2	0.001
Ga	23.3	0.000	Y	20.4	0.000
Gd	22.7	0.000	Zn	19.9	0.000
K	20.4	0.000	Zr	24.0	0.000
Li	21.2	0.000			

Table A7.22: KW-H results for the Bolingey Stream study catchment.

Property	H-value	p-value	Property	H-value	p-value
Al	12.6	0.006	Pb	25.1	0.000
As	20.1	0.000	Pr	23.6	0.000
Bi	22.2	0.000	Sb	21.1	0.000
Cr	6.3	0.097	Sc	19.2	0.000
Dy	31.4	0.000	Sm	27.1	0.000
Er	24.7	0.000	Sr	29.0	0.000
Gd	30.4	0.000	Tb	21.7	0.000
Ge	26.2	0.000	Ti	28.1	0.000
Ho	30.9	0.000	Tl	22.3	0.000
La	27.2	0.000	Tm	26.3	0.000
Li	8.1	0.044	U	28.8	0.000
Mg	11.7	0.009	V	26.3	0.000
Na	16.8	0.001	Y	28.4	0.000
Nd	25.5	0.000	Yb	22.3	0.000

Table A7.23: KW-H results for the Egglestone Beck study catchment.

Property	H-value	p-value	Property	H-value	p-value
Al	10.2	0.017	Mg	15.2	0.002
As	13.5	0.004	Na	3.4	0.331
Cs	15.1	0.002	Pb	9.5	0.023
Cu	13.4	0.004	Rb	11.0	0.012
Dy	15.8	0.001	Sb	10.5	0.015
Eu	14.5	0.002	Sc	16.8	0.001
Fe	18.1	0.000	Sr	5.7	0.127
Ga	13.4	0.004	Tm	17.2	0.001
Gd	17.7	0.001	V	10.9	0.012
K	10.2	0.017	Y	14.2	0.003
Li	7.1	0.070	Zr	16.8	0.001

Table A7.24: KW-H results for the Hayle study catchment.

Property	H-value	p-value	Property	H-value	p-value
As	18.0	0.001	Na	36.7	0.000
Ba	24.0	0.000	Pb	27.2	0.000
Bi	19.0	0.001	Sb	23.7	0.000
Cr	16.1	0.003	Sr	27.6	0.000
Dy	20.7	0.000	Tb	20.1	0.000
Er	21.3	0.000	Ti	31.7	0.000
Fe	28.3	0.000	Tm	22.6	0.000
Ga	24.2	0.000	V	37.3	0.000
Ho	22.1	0.000	Y	27.1	0.000
Li	28.2	0.000	Yb	22.7	0.000
Lu	21.8	0.000	Zr	33.2	0.000
Mg	32.4	0.000			

Table A7.25: KW-H results for the Hudeshope Beck study catchment.

Property	H-value	p-value	Property	H-value	p-value
Al	25.991	0.000	Na	23.828	0.000
As	33.681	0.000	Ni	23.303	0.000
Cs	34.061	0.000	Rb	36.629	0.000
Cu	19.810	0.001	Sc	21.711	0.000
Ga	24.231	0.000	Sr	23.866	0.000
K	34.056	0.000	Ti	27.082	0.000
Li	20.448	0.000	Tm	22.686	0.000
Mg	30.624	0.000	V	24.156	0.000

Table A7.26: KW-H results for the Mardle study catchment.

Property	H-value	p-value	Property	H-value	p-value
Al	28.3	0.000	Mg	30.1	0.000
As	27.1	0.000	Mo	27.0	0.000
Ba	26.5	0.000	Na	21.9	0.000
Bi	26.6	0.000	Pb	29.5	0.000
Cd	21.7	0.000	Rb	29.5	0.000
Ce	20.9	0.000	Sb	25.6	0.000
Co	25.5	0.000	Sc	26.8	0.000
Cs	21.1	0.000	Sn	27.8	0.000
Cu	25.0	0.000	Sr	31.4	0.000
Dy	26.9	0.000	Ti	14.8	0.002
Er	28.7	0.000	Tl	34.2	0.000
Eu	25.4	0.000	U	21.9	0.000
Fe	25.9	0.000	V	15.5	0.001
Ga	27.9	0.000	Yb	28.3	0.000
K	27.1	0.000	Zr	25.0	0.000
Lu	28.5	0.000			

Table A7.27: KW-H results for the Nant Magwr study catchment.

Property	H-value	p-value	Property	H-value	p-value
As	25.3	0.000	Sb	28.3	0.000
Co	25.0	0.000	Sc	21.1	0.000
Dy	25.0	0.000	Sm	25.8	0.000
Eu	32.0	0.000	Sn	23.6	0.000
Ga	29.9	0.000	Sr	20.0	0.001
Gd	26.1	0.000	Tb	30.7	0.000
Ge	26.3	0.000	Tl	25.4	0.000
La	28.9	0.000	Tm	18.8	0.001
Mo	36.7	0.000	Y	24.1	0.000
Pr	30.5	0.000			

Table A7.28: KW-H results for the Porthleven Stream study catchment.

Property	H-value	p-value	Property	H-value	p-value
Ce	11.7	0.019	Nd	11.8	0.019
Cr	6.3	0.177	Ni	6.7	0.152
Dy	21.3	0.000	Pr	12.6	0.013
Er	11.7	0.020	Sm	12.7	0.013
Eu	11.5	0.022	Sn	13.3	0.010
Gd	13.2	0.010	Tb	15.0	0.005
Ge	14.5	0.006	Tl	22.7	0.000
Ho	16.1	0.003	Tm	14.7	0.005
La	11.9	0.018	U	14.8	0.005
Li	7.3	0.122	V	27.5	0.000
Lu	26.2	0.000	Y	20.2	0.000
Mg	5.9	0.206	Yb	11.2	0.024
Na	18.9	0.001	Zr	19.5	0.001

Table A7.29: KW-H results for the Rea Brook study catchment.

Property	H-value	p-value	Property	H-value	p-value
Al	23.4	0.000	Mo	23.7	0.000
Ba	22.9	0.000	Nd	29.1	0.000
Ce	25.9	0.000	Pb	22.5	0.000
Cr	16.9	0.002	Pr	27.3	0.000
Cu	21.3	0.000	Sm	30.2	0.000
Dy	32.2	0.000	Sn	31.2	0.000
Er	31.6	0.000	Sr	25.7	0.000
Eu	28.7	0.000	Tb	27.8	0.000
Ga	34.2	0.000	Ti	29.3	0.000
Gd	30.3	0.000	Tm	29.5	0.000
Ge	37.2	0.000	V	32.0	0.000
Ho	30.2	0.000	Y	32.5	0.000
La	27.0	0.000	Yb	30.4	0.000
Lu	30.2	0.000	Zn	21.9	0.000
Mg	29.5	0.000			

Table A7.30: KW-H results for the Red Tarn Beck study catchment.

Property	H-value	p-value	Property	H-value	p-value
As	20.3	0.000	Sm	13.7	0.003
Bi	23.0	0.000	Sn	17.3	0.001
Ga	15.4	0.002	Sr	17.9	0.000
Gd	14.3	0.002	Tb	4.7	0.192
Hf	23.6	0.000	Tm	23.3	0.000
Mo	22.4	0.000	U	15.8	0.001
Nd	11.8	0.008	Zr	23.1	0.000

Table A7.31: KW-H results for the River Burn (Tavy) study catchment.

Property	H-value	p-value	Property	H-value	p-value
Ce	35.3	0.000	Mo	33.7	0.000
Cs	21.0	0.000	Nd	37.9	0.000
Cu	33.4	0.000	Pr	37.7	0.000
Dy	35.5	0.000	Sb	25.3	0.000
Er	34.7	0.000	Sc	31.1	0.000
Eu	35.4	0.000	Sm	39.7	0.000
Fe	31.0	0.000	Sn	29.8	0.000
Gd	39.3	0.000	Sr	23.1	0.000
Ge	40.8	0.000	Tl	32.6	0.000
Hf	27.6	0.000	Tm	36.2	0.000
K	24.2	0.000	U	33.7	0.000
La	37.2	0.000	Y	36.0	0.000
Lu	36.6	0.000	Yb	35.0	0.000

Table A7.32: KW-H results for the River East Allen study catchment.

Property	H-value	p-value	Property	H-value	p-value
As	15.0	0.005	Na	29.6	0.000
Bi	20.1	0.000	Ni	9.2	0.057
Cs	37.8	0.000	Sr	26.7	0.000
Cu	17.6	0.001	Tb	17.0	0.002
Dy	17.4	0.002	Ti	38.7	0.000
Er	13.9	0.008	Tm	13.2	0.010
Gd	22.0	0.000	V	35.0	0.000
Hf	15.6	0.004	Y	14.5	0.006
Ho	14.6	0.006	Yb	12.8	0.012
Mg	34.1	0.000	Zr	14.7	0.005

Table A7.33: KW-H results for the River Greta study catchment.

Property	H-value	p-value	Property	H-value	p-value
As	31.6	0.000	Li	19.5	0.000
Ba	18.3	0.000	Mg	22.0	0.000
Bi	27.5	0.000	Mo	28.1	0.000
Ce	29.6	0.000	Nd	28.0	0.000
Cs	26.8	0.000	Pb	21.9	0.000
Cu	15.8	0.001	Pr	26.7	0.000
Dy	22.1	0.000	Sb	27.7	0.000
Eu	19.2	0.000	Sm	26.4	0.000
Fe	30.5	0.000	Sn	22.3	0.000
Ga	30.9	0.000	Sr	26.6	0.000
Gd	26.4	0.000	Ti	30.8	0.000
Ge	27.8	0.000	V	31.5	0.000
La	27.8	0.000	Y	11.2	0.011

Table A7.34: KW-H results for the River South Tyne study catchment.

Property	H-value	p-value	Property	H-value	p-value
As	31.6	0.000	Pb	22.5	0.000
Cd	28.2	0.000	Rb	31.8	0.000
Co	29.3	0.000	Sc	24.7	0.000
Cs	31.7	0.000	Sr	18.3	0.001
Ga	19.8	0.001	Ti	27.9	0.000
K	26.9	0.000	U	22.2	0.000
Mg	23.0	0.000	V	20.4	0.000
Mn	26.7	0.000	Zn	27.4	0.000
Na	22.4	0.000	Zr	25.2	0.000
Ni	33.0	0.000			

Table A7.35: KW-H results for the St Lawrence Stream study catchment.

Property	H-value	p-value	Property	H-value	p-value
As	30.6	0.000	Mn	32.0	0.000
Bi	19.8	0.001	Mo	19.7	0.001
Co	29.0	0.000	Nd	28.1	0.000
Cr	26.2	0.000	Pr	27.9	0.000
Cu	25.9	0.000	Rb	31.6	0.000
Dy	27.5	0.000	Sb	37.1	0.000
Er	24.7	0.000	Sc	22.5	0.000
Eu	29.3	0.000	Sm	28.1	0.000
Fe	32.1	0.000	Sr	37.9	0.000
Gd	28.9	0.000	Tb	27.3	0.000
Ge	41.8	0.000	Ti	26.2	0.000
Ho	26.4	0.000	Tl	37.7	0.000
K	24.2	0.000	Tm	26.8	0.000
La	27.0	0.000	U	31.6	0.000
Li	24.6	0.000	V	36.3	0.000
Lu	27.1	0.000	Y	26.6	0.000
Mg	30.9	0.000	Yb	26.9	0.000

Table A7.36: KW-H results for the Wye study catchment.

Property	H-value	p-value	Property	H-value	p-value
Al	18.8	0.000	Lu	18.3	0.000
Bi	13.9	0.001	Mn	18.1	0.000
Cd	17.8	0.000	Rb	16.4	0.000
Cs	14.6	0.001	Sb	16.7	0.000
Dy	17.3	0.000	Sc	17.2	0.000
Er	18.2	0.000	Sr	18.3	0.000
Eu	16.5	0.000	Tb	14.7	0.001
Gd	10.7	0.005	Tm	18.2	0.000
Ho	18.1	0.000	Y	18.1	0.000
K	17.2	0.000	Yb	18.3	0.000
Li	17.8	0.000	Zn	18.1	0.000

Table A7.37: Highest ranked property loadings provided by the outputs of the PCA for the Afon Ystwyth study catchment.

Property	PC-1[a]	Property	PC-2[b]
Pb	1.000	Cu	1.000
Cu	0.012	Pb	0.012
Sb	0.004	Sb	0.011
As	0.003	Sn	0.006
Ga	0.000	Ga	0.006
V	0.000	As	0.004
Zr	0.000	Sm	0.002
Sn	0.000	V	0.002
Sm	0.000	Cs	0.001
Tl	0.000	Sc	0.001
Sc	0.000	Tl	0.001
Cs	0.000	U	0.000
U	0.000	Zr	0.000
Hf	0.000	Hf	0.000
VE%	100.0	VE%	0.0

a Principal Component 1; b Principal Component 2; VE % variance explained

Table A7.38: Highest ranked property loadings provided by the outputs of the PCA for the Arkle Beck study catchment.

Property	PC-1[a]	Property	PC-2[b]
Zn	0.998	K	0.998
K	0.066	Zn	0.066
Ni	0.002	Cu	0.007
Cu	0.002	Li	0.005
Zr	0.000	Rb	0.004
V	0.000	V	0.003
As	0.000	Zr	0.002
Sb	0.000	Ni	0.002
Sn	0.000	Cs	0.001
Li	0.000	Sn	0.001
Rb	0.000	Sc	0.000
Mo	0.000	As	0.000
U	0.000	Sb	0.000
Bi	0.000	Mo	0.000
Cs	0.000	Bi	0.000
Sc	0.000	U	0.000
Er	0.000	Er	0.000
Yb	0.000	Yb	0.000
Ho	0.000	Eu	0.000
Eu	0.000	Ho	0.000
Tm	0.000	Tm	0.000
VE%	90.1	VE%	9.9

a Principal Component 1; b Principal Component 2; VE % variance explained

Table A7.39: Highest ranked property loadings provided by the outputs of the PCA for the Bedburn Beck study catchment.

Property	PC-1[a]	Property	PC-2[b]
Al	0.835	Pb	0.900
K	0.431	Al	0.402
Pb	0.336	Zn	0.149
Zn	0.065	Na	0.060
Na	0.008	K	0.054
Li	0.002	Ti	0.010
Rb	0.002	Sb	0.005
Y	0.001	Y	0.003
Sb	0.001	Cu	0.002
Sr	0.001	Li	0.002
Cu	0.001	Zr	0.001
Zr	0.001	As	0.001
Ti	0.000	Eu	0.000
As	0.000	Gd	0.000
Cs	0.000	Dy	0.000
Eu	0.000	Sr	0.000
Ga	0.000	Er	0.000
Gd	0.000	Rb	0.000
Dy	0.000	Sc	0.000
Sc	0.000	Ga	0.000
Er	0.000	Cs	0.000
VE%	89.1	**VE%**	9.8

a Principal Component 1; b Principal Component 2; VE % variance explained

Table A7.40: Highest ranked property loadings provided by the outputs of the PCA for the Bolingey Stream study catchment.

Property	PC-1[a]	Property	PC-2[b]
Pb	0.717	Pb	0.696
Al	0.675	Al	0.681
Mg	0.176	Mg	0.226
As	0.011	As	0.010
Na	0.007	Li	0.002
Li	0.003	Sb	0.002
Sb	0.002	Ti	0.002
Cr	0.001	Cr	0.002
Sr	0.001	Na	0.001
Ti	0.001	Sr	0.001
Y	0.000	La	0.000
V	0.000	Nd	0.000
La	0.000	Y	0.000
Nd	0.000	V	0.000
Ge	0.000	Ge	0.000
Bi	0.000	Sc	0.000
U	0.000	U	0.000
Sm	0.000	Bi	0.000
Pr	0.000	Pr	0.000
Tl	0.000	Sm	0.000
Dy	0.000	Dy	0.000
Er	0.000	Gd	0.000
Sc	0.000	Ho	0.000
Yb	0.000	Tl	0.000
Ho	0.000	Er	0.000
Gd	0.000	Yb	0.000
Tb	0.000	Tb	0.000
Tm	0.000	Tm	0.000
VE%	65.5	VE%	30.5

a Principal Component 1; b Principal Component 2; VE % variance explained

Table A7.41: Highest ranked property loadings provided by the outputs of the PCA for the Egglestone Beck study catchment.

Property	PC-1[a]	Property	PC-2[b]
Al	0.776	Fe	0.651
Fe	0.445	Al	0.548
K	0.322	Pb	0.511
Pb	0.310	Mg	0.090
Na	0.004	K	0.074
Li	0.002	Na	0.021
Rb	0.002	Sb	0.006
Cu	0.001	Cu	0.004
Mg	0.001	Sr	0.002
Sb	0.001	Li	0.002
Sr	0.001	Y	0.001
Y	0.001	As	0.001
As	0.001	Zr	0.001
Zr	0.001	V	0.001
V	0.000	Rb	0.001
Ga	0.000	Eu	0.000
Cs	0.000	Ga	0.000
Sc	0.000	Dy	0.000
Gd	0.000	Cs	0.000
Eu	0.000	Gd	0.000
Dy	0.000	Sc	0.000
Tm	0.000	Tm	0.000
VE%	69.1	VE%	24.0

a Principal Component 1; b Principal Component 2; VE % variance explained

Table A7.42: Highest ranked property loadings provided by the outputs of the PCA for the Hayle study catchment.

Property	PC-1[a]	Property	PC-2[b]
Fe	0.996	Mg	0.985
Mg	0.090	Ti	0.135
As	0.020	Fe	0.087
Pb	0.013	As	0.062
Ti	0.003	Na	0.029
Bi	0.003	Bi	0.013
Na	0.003	Pb	0.009
Li	0.001	V	0.007
Sr	0.001	Li	0.005
Ba	0.000	Cr	0.005
V	0.000	Ba	0.001
Cr	0.000	Sr	0.001
Y	0.000	Y	0.000
Ga	0.000	Ga	0.000
Sb	0.000	Zr	0.000
Zr	0.000	Sb	0.000
Dy	0.000	Dy	0.000
Er	0.000	Er	0.000
Yb	0.000	Yb	0.000
Ho	0.000	Tb	0.000
Tb	0.000	Lu	0.000
Tm	0.000	Tm	0.000
Lu	0.000	Ho	0.000
VE%	99.4	VE%	0.6

a Principal Component 1; b Principal Component 2; VE % variance explained

Table A7.43: Highest ranked property loadings provided by the outputs of the PCA for the Hudeshope Beck study catchment.

Property	PC-1[a]	Property	PC-2[b]
Al	0.879	Mg	0.933
K	0.449	Na	0.283
Mg	0.153	Ti	0.146
Na	0.039	Al	0.122
Ti	0.025	K	0.112
Sr	0.004	Sr	0.022
Rb	0.002	Cu	0.010
Li	0.002	V	0.006
Cu	0.001	Li	0.002
As	0.000	Rb	0.001
V	0.000	Cs	0.001
Cs	0.000	Ni	0.000
Ga	0.000	As	0.000
Sc	0.000	Tm	0.000
Ni	0.000	Sc	0.000
Tm	0.000	Ga	0.000
VE%	93.7	VE%	3.9

a Principal Component 1; b Principal Component 2; VE % variance explained

Table A7.44: Highest ranked property loadings provided by the outputs of the PCA for the Mardle study catchment.

Property	PC-1[a]	Property	PC-2[b]
Fe	0.965	Al	0.879
Al	0.170	K	0.422
As	0.135	Fe	0.216
K	0.134	Mg	0.039
Cu	0.044	Na	0.017
Mg	0.031	Bi	0.015
Bi	0.008	Pb	0.013
Pb	0.006	Cu	0.009
Na	0.004	As	0.005
Ti	0.002	Ba	0.004
Co	0.001	Rb	0.004
Sn	0.001	Sn	0.004
Ba	0.001	Sr	0.002
Rb	0.001	Ti	0.002
Sr	0.001	Sb	0.001
Sb	0.001	Zr	0.001
Zr	0.000	Ce	0.001
Mo	0.000	Ga	0.000
Ga	0.000	Cs	0.000
V	0.000	V	0.000
Sc	0.000	Sc	0.000
Ce	0.000	Mo	0.000
Cs	0.000	U	0.000
Cd	0.000	Tl	0.000
Tl	0.000	Dy	0.000
U	0.000	Co	0.000
Eu	0.000	Yb	0.000
Yb	0.000	Er	0.000
Er	0.000	Eu	0.000
Lu	0.000	Cd	0.000
Dy	0.000	Lu	0.000
VE%	91.4	VE%	7.9

a Principal Component 1; b Principal Component 2; VE % variance explained

Table A7.45: Highest ranked property loadings provided by the outputs of the PCA for the Nant Magwr study catchment.

Property	PC-1[a]	Property	PC-2[b]
Sb	0.860	La	0.784
La	0.428	Sb	0.438
As	0.154	Pr	0.265
Pr	0.144	Ga	0.175
Co	0.101	Ge	0.159
Ge	0.088	Sr	0.152
Sm	0.081	Sm	0.141
Gd	0.052	As	0.119
Ga	0.046	Gd	0.088
Mo	0.033	Eu	0.032
Y	0.029	Y	0.032
Sr	0.025	Dy	0.018
Eu	0.018	Co	0.011
Sn	0.016	Tb	0.008
Dy	0.012	Sc	0.008
Tl	0.012	Sn	0.006
Sc	0.009	Tl	0.003
Tb	0.005	Mo	0.002
Tm	0.000	Tm	0.000
VE%	66.8	VE%	28.7

a Principal Component 1; b Principal Component 2; VE % variance explained

Table A7.46: Highest ranked property loadings provided by the outputs of the PCA for the Porthleven Stream study catchment.

Property	PC-1[a]	Property	PC-2[b]
Mg	0.999	Na	0.987
Na	0.045	Sn	0.111
Ni	0.005	Li	0.089
Sn	0.005	Mg	0.044
Cr	0.004	Cr	0.043
V	0.003	Ce	0.033
Ce	0.003	V	0.013
Li	0.002	U	0.013
La	0.001	La	0.013
Nd	0.001	Nd	0.012
Pr	0.000	Y	0.008
Ge	0.000	Ni	0.007
Sm	0.000	Zr	0.005
Y	0.000	Pr	0.003
Gd	0.000	Ge	0.001
Dy	0.000	Sm	0.001
Eu	0.000	Dy	0.001
Er	0.000	Ho	0.001
Zr	0.000	Eu	0.001
Yb	0.000	Tl	0.001
Ho	0.000	Gd	0.001
Tb	0.000	Tb	0.000
U	0.000	Lu	0.000
Tm	0.000	Er	0.000
Tl	0.000	Tm	0.000
Lu	0.000	Yb	0.000
VE%	99.6	VE%	0.3

a Principal Component 1; b Principal Component 2; VE % variance explained

Table A7.47: Highest ranked property loadings provided by the outputs of the PCA for the Rea Brook study catchment.

Property	PC-1[a]	Property	PC-2[b]
Pb	0.837	Al	0.849
Zn	0.458	Mg	0.510
Mg	0.279	Pb	0.080
Ba	0.099	Ti	0.080
Al	0.048	Zn	0.068
Ti	0.011	Ba	0.036
Sr	0.008	Cu	0.004
Ce	0.002	Sr	0.004
Y	0.001	V	0.004
V	0.001	Sn	0.001
Nd	0.001	Ce	0.001
La	0.001	Y	0.000
Cr	0.000	Cr	0.000
Gd	0.000	Nd	0.000
Sm	0.000	La	0.000
Ga	0.000	Ga	0.000
Ge	0.000	Gd	0.000
Dy	0.000	Eu	0.000
Pr	0.000	Mo	0.000
Eu	0.000	Sm	0.000
Cu	0.000	Pr	0.000
Sn	0.000	Dy	0.000
Mo	0.000	Er	0.000
Er	0.000	Yb	0.000
Yb	0.000	Ho	0.000
Tb	0.000	Ge	0.000
Ho	0.000	Tb	0.000
Tm	0.000	Tm	0.000
Lu	0.000	Lu	0.000
VE%	70.0	VE%	18.3

a Principal Component 1; b Principal Component 2; VE % variance explained

Table A7.48: Highest ranked property loadings provided by the outputs of the PCA for the Red Tarn Beck study catchment.

Property	PC-1[a]	Property	PC-2[b]
Sr	0.892	As	0.895
As	0.441	Sr	0.442
Nd	0.088	Sn	0.063
Ga	0.025	Bi	0.014
Sn	0.017	Zr	0.011
Sm	0.017	Nd	0.010
Mo	0.016	Ga	0.008
Gd	0.014	Mo	0.006
Hf	0.011	U	0.004
U	0.004	Tb	0.002
Zr	0.003	Gd	0.002
Tb	0.002	Hf	0.001
Bi	0.002	Tm	0.001
Tm	0.000	Sm	0.000
VE%	66.3	VE%	32.0

a Principal Component 1; b Principal Component 2; VE % variance explained

Table A7.49: Highest ranked property loadings provided by the outputs of the PCA for the River Burn (Tavy) study catchment.

Property	PC-1[a]	Property	PC-2[b]
Fe	1.000	K	0.999
Cu	0.004	Cu	0.041
K	0.004	Sr	0.005
Y	0.000	Fe	0.004
Ce	0.000	La	0.002
Nd	0.000	Ce	0.002
La	0.000	Sb	0.001
Sr	0.000	Y	0.001
Ge	0.000	Mo	0.001
Mo	0.000	Sn	0.001
Gd	0.000	Nd	0.000
Dy	0.000	Sc	0.000
Sm	0.000	Pr	0.000
Pr	0.000	Ge	0.000
Sn	0.000	Dy	0.000
Sc	0.000	Sm	0.000
Er	0.000	Er	0.000
Yb	0.000	Lu	0.000
Sb	0.000	Yb	0.000
Cs	0.000	Eu	0.000
Eu	0.000	Tm	0.000
U	0.000	Cs	0.000
Lu	0.000	U	0.000
Tm	0.000	Hf	0.000
Tl	0.000	Tl	0.000
Hf	0.000	Gd	0.000
VE%	100.0	VE%	0.0

a Principal Component 1; b Principal Component 2; VE % variance explained

Table A7.50: Highest ranked property loadings provided by the outputs of the PCA for the River East Allen study catchment.

Property	PC-1[a]	Property	PC-2[b]
Mg	0.912	Na	0.912
Na	0.390	Mg	0.401
Ti	0.128	Ti	0.081
Sr	0.019	Sr	0.030
V	0.003	Cu	0.014
Cu	0.003	Zr	0.007
Zr	0.001	Ni	0.006
Cs	0.001	Y	0.005
As	0.001	As	0.002
Ni	0.001	Cs	0.001
Y	0.000	V	0.000
Gd	0.000	Dy	0.000
Bi	0.000	Hf	0.000
Dy	0.000	Gd	0.000
Hf	0.000	Bi	0.000
Er	0.000	Er	0.000
Yb	0.000	Ho	0.000
Ho	0.000	Tb	0.000
Tm	0.000	Yb	0.000
Tb	0.000	Tm	0.000
VE%	97.4	VE%	2.2

a Principal Component 1; b Principal Component 2; VE % variance explained

Table A7.51: Highest ranked property loadings provided by the outputs of the PCA for the River Greta study catchment.

Property	PC-1[a]	Property	PC-2[b]
Fe	0.964	Pb	0.916
Pb	0.256	Mg	0.330
Mg	0.064	Fe	0.221
Ti	0.016	Ti	0.044
As	0.009	Ba	0.043
Ba	0.005	As	0.017
Sr	0.004	Sr	0.014
Ce	0.002	Cu	0.006
Sb	0.001	Sb	0.005
La	0.001	V	0.004
Li	0.001	Sn	0.002
Nd	0.001	Li	0.002
V	0.001	Ce	0.001
Sn	0.000	La	0.001
Ga	0.000	Nd	0.000
Cu	0.000	Mo	0.000
Pr	0.000	Y	0.000
Ge	0.000	Cs	0.000
Sm	0.000	Gd	0.000
Cs	0.000	Sm	0.000
Gd	0.000	Dy	0.000
Mo	0.000	Bi	0.000
Eu	0.000	Pr	0.000
Y	0.000	Ge	0.000
Dy	0.000	Ga	0.000
Bi	0.000	Eu	0.000
VE%	96.1	VE%	3.0

a Principal Component 1; b Principal Component 2; VE % variance explained

Table A7.52: Highest ranked property loadings provided by the outputs of the PCA for the River South Tyne study catchment.

Property	PC-1[a]	Property	PC-2[b]
Zn	0.841	Pb	0.699
Pb	0.495	Mg	0.494
K	0.156	Zn	0.397
Mg	0.119	K	0.326
Mn	0.090	Mn	0.046
Na	0.009	Na	0.026
Ti	0.008	Ti	0.025
As	0.004	Sr	0.004
Cd	0.002	As	0.003
Ni	0.001	Ni	0.002
Rb	0.001	Rb	0.002
Co	0.001	Cd	0.001
Ga	0.000	Co	0.001
Cs	0.000	V	0.000
Sr	0.000	Cs	0.000
Zr	0.000	Zr	0.000
Sc	0.000	Ga	0.000
V	0.000	Sc	0.000
U	0.000	U	0.000
VE%	86.2	VE%	7.6

a Principal Component 1; b Principal Component 2; VE % variance explained

Table A7.53: Highest ranked property loadings provided by the outputs of the PCA for the St Lawrence Stream study catchment.

Property	PC-1[a]	Property	PC-2[b]
Fe	0.999	Mg	0.998
Mn	0.041	Ti	0.052
Mg	0.020	Fe	0.021
Cu	0.016	Mn	0.015
K	0.010	Cu	0.010
As	0.005	K	0.009
Co	0.001	Li	0.005
Ti	0.001	Cr	0.004
Li	0.000	V	0.003
Nd	0.000	Sr	0.003
La	0.000	As	0.003
Y	0.000	La	0.001
Rb	0.000	Nd	0.001
Cr	0.000	Rb	0.001
Ge	0.000	Co	0.000
V	0.000	Y	0.000
Sr	0.000	Pr	0.000
Pr	0.000	Sb	0.000
Gd	0.000	Sc	0.000
Sm	0.000	Sm	0.000
Dy	0.000	U	0.000
U	0.000	Gd	0.000
Sb	0.000	Ge	0.000
Er	0.000	Bi	0.000
Bi	0.000	Mo	0.000
Eu	0.000	Dy	0.000
Tl	0.000	Eu	0.000
Yb	0.000	Tl	0.000
Tb	0.000	Er	0.000
Mo	0.000	Yb	0.000
Ho	0.000	Tb	0.000
Sc	0.000	Ho	0.000
Tm	0.000	Tm	0.000
Lu	0.000	Lu	0.000
VE%	99.5	VE%	0.4

a Principal Component 1; b Principal Component 2; VE % variance explained

Table A7.54: Highest ranked property loadings provided by the outputs of the PCA for the Wye study catchment.

Property	PC-1[a]	Property	PC-2[b]
Zn	0.788	Al	0.779
Al	0.510	Zn	0.593
Mn	0.344	Mn	0.197
K	0.034	K	0.059
Cd	0.004	Cd	0.003
Li	0.001	Li	0.002
Y	0.001	Sb	0.001
Sr	0.001	Sr	0.001
Sb	0.001	Y	0.001
Gd	0.000	Rb	0.000
Eu	0.000	Cs	0.000
Dy	0.000	Eu	0.000
Rb	0.000	Dy	0.000
Sc	0.000	Gd	0.000
Cs	0.000	Sc	0.000
Er	0.000	Er	0.000
Yb	0.000	Yb	0.000
Tb	0.000	Tb	0.000
Ho	0.000	Ho	0.000
Tm	0.000	Tm	0.000
Lu	0.000	Lu	0.000
Bi	0.000	Bi	0.000
VE%	89.3	VE%	9.0

a Principal Component 1; b Principal Component 2; VE % variance explained

Table A7.55: The final composite signatures selected using KW-H and PCA for the Afon Ystwyth study catchment.

KW-H			PCA		
Property	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**
As	78	1.43	As	78	1.79
Ga	58	1.07	Cu	43	1.00
Sn	54	1.00	Ga	58	1.34
Tl	65	1.21	Pb	65	1.50
V	56	1.04	Sb	63	1.44
Zr	58	1.06	Sn	54	1.25
Total[3]	88		Total[3]	75	

[1] % source type end member samples classified correctly by individual properties

[2] tracer discriminatory weighting used in the mass balance modelling

[3] % source type end member samples classified correctly by composite signature

Table A7.56: The final composite signatures selected using KW-H and PCA for the Arkle Beck study catchment.

KW-H			PCA		
Property	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**
Bi	78	1.10	K	75	1.47
Ho	71	1.00	Li	61	1.20
Sn	89	1.26	Ni	54	1.05
V	80	1.13	Zn	51	1.00
Total[3]	93		Total[3]	68	

[1] % source type end member samples classified correctly by individual properties

[2] tracer discriminatory weighting used in the mass balance modelling

[3] % source type end member samples classified correctly by composite signature

Table A7.57: The final composite signatures selected using KW-H and PCA for the Bedburn Beck study catchment.

KW-H			PCA		
Property	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**
Cs	71	1.19	Al	56	1.21
Cu	60	1.00	K	62	1.33
Ga	72	1.20	Na	46	1.00
Rb	66	1.10	Pb	61	1.32
Zr	74	1.25	Zn	47	1.01
Total[3]	85		Total[3]	73	

[1] % source type end member samples classified correctly by individual properties

[2] tracer discriminatory weighting used in the mass balance modelling

[3] % source type end member samples classified correctly by composite signature

Table A7.58: The final composite signatures selected using KW-H and PCA for the Bolingey Stream study catchment.

KW-H			PCA		
Property	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**
Dy	73	1.33	Al	47	1.28
Gd	61	1.10	As	44	1.21
Ho	69	1.26	Mg	48	1.32
Sr	55	1.00	Na	47	1.29
U	58	1.06	Pb	36	1.00
Total[3]	89		Total[3]	72	

[1] % source type end member samples classified correctly by individual properties

[2] tracer discriminatory weighting used in the mass balance modelling

[3] % source type end member samples classified correctly by composite signature

Table A7.59: The final composite signatures selected using KW-H and PCA for the Egglestone Beck study catchment.

KW-H			PCA		
Property	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**
Dy	66	1.19	Al	49	1.39
Fe	67	1.21	Fe	67	1.90
Gd	75	1.35	K	49	1.37
Mg	56	1.00	Li	51	1.45
Sc	58	1.05	Mg	56	1.57
Tm	73	1.31	Na	35	1.00
Zr	63	1.13	Pb	48	1.35
Total[3]	95		Total[3]	92	

[1] % source type end member samples classified correctly by individual properties

[2] tracer discriminatory weighting used in the mass balance modelling

[3] % source type end member samples classified correctly by composite signature

Table A7.60: The final composite signatures selected using KW-H and PCA for the Hayle study catchment.

KW-H			PCA		
Property	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**
Fe	48	1.09	As	47	1.21
Li	46	1.05	Bi	39	1.00
Mg	53	1.20	Fe	48	1.23
Na	70	1.59	Li	46	1.19
Sr	44	1.00	Mg	53	1.36
Ti	59	1.33	Na	70	1.79
V	67	1.52	Pb	47	1.20
Zr	48	1.09	Ti	59	1.50
Total[3]	90		Total[3]	84	

[1] % source type end member samples classified correctly by individual properties

[2] tracer discriminatory weighting used in the mass balance modelling

[3] % source type end member samples classified correctly by composite signature

Table A7.61: The final composite signatures selected using KW-H and PCA for the Hudeshope Beck study catchment.

KW-H			PCA		
Property	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**
As	48	1.00	Al	48	1.07
Cs	63	1.31	K	57	1.27
K	57	1.18	Mg	60	1.35
Mg	60	1.25	Na	54	1.21
Rb	60	1.25	Sr	45	1.00
Ti	58	1.19	Ti	58	1.29
Total[3]	82		Total[3]	82	

[1] % source type end member samples classified correctly by individual properties

[2] tracer discriminatory weighting used in the mass balance modelling

[3] % source type end member samples classified correctly by composite signature

Table A7.62: The final composite signatures selected using KW-H and PCA for the Mardle study catchment.

KW-H			PCA		
Property	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**
Al	68	1.33	Al	68	1.67
As	51	1.00	As	51	1.26
Er	68	1.33	Ba	61	1.49
Ga	71	1.38	Bi	49	1.19
Lu	68	1.32	Cu	51	1.24
Mg	65	1.27	Fe	53	1.30
Pb	64	1.24	K	50	1.23
Rb	68	1.33	Mg	65	1.59
Sn	59	1.15	Na	41	1.00
Sr	60	1.16	Pb	64	1.56
Tl	69	1.34	Sn	59	1.44
Yb	68	1.33	Ti	43	1.04
Total[3]	95		Total[3]	90	

[1] % source type end member samples classified correctly by individual properties

[2] tracer discriminatory weighting used in the mass balance modelling

[3] % source type end member samples classified correctly by composite signature

Table A7.63: The final composite signatures selected using KW-H and PCA for the Nant Magwr study catchment.

KW-H			PCA		
Property	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**
Eu	44	1.12	As	52	1.31
Ga	39	1.00	Co	46	1.17
La	41	1.03	Ga	39	1.00
Mo	67	1.70	La	41	1.03
Pr	48	1.22	Pr	48	1.22
Sb	48	1.22	Sb	48	1.22
Tb	56	1.43	Sm	54	1.38
Total[3]	74		Total[3]	80	

[1] % source type end member samples classified correctly by individual properties

[2] tracer discriminatory weighting used in the mass balance modelling

[3] % source type end member samples classified correctly by composite signature

Table A7.64: The final composite signatures selected using KW-H and PCA for the Porthleven Stream study catchment.

KW-H			PCA		
Property	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**
Dy	55	1.13	Cr	37	1.14
Lu	51	1.04	Li	37	1.14
Na	55	1.14	Mg	53	1.65
Tl	49	1.01	Na	55	1.71
V	66	1.36	Ni	39	1.21
Y	57	1.17	Sn	32	1.00
Zr	49	1.00	V	66	2.05
Total[3]	100		Total[3]	90	

[1] % source type end member samples classified correctly by individual properties

[2] tracer discriminatory weighting used in the mass balance modelling

[3] % source type end member samples classified correctly by composite signature

Table A7.65: The final composite signatures selected using KW-H and PCA for the Rea Brook study catchment.

KW-H			PCA		
Property	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**
Dy	61	1.21	Al	44	1.23
Er	59	1.17	Ba	36	1.00
Ga	54	1.07	Ce	58	1.60
Gd	63	1.25	Mg	56	1.56
Ge	70	1.39	Pb	50	1.39
Lu	62	1.23	Sr	62	1.72
Sn	51	1.00	Ti	53	1.47
V	60	1.19	V	60	1.67
Y	68	1.35	Y	68	1.90
Yb	58	1.15	Zn	54	1.49
Total3	94		Total[3]	92	

[1] % source type end member samples classified correctly by individual properties

[2] tracer discriminatory weighting used in the mass balance modelling

[3] % source type end member samples classified correctly by composite signature

Table A7.66: The final composite signatures selected using KW-H and PCA for the Red Tarn Beck study catchment.

KW-H			PCA		
Property	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**
As	65	1.00	As	65	1.22
Bi	69	1.06	Ga	58	1.09
Hf	83	1.28	Nd	56	1.05
Mo	85	1.30	Sm	59	1.12
Tm	70	1.08	Sn	53	1.00
Zr	72	1.11	Sr	56	1.06
Total[3]	82		Total[3]	87	

[1] % source type end member samples classified correctly by individual properties

[2] tracer discriminatory weighting used in the mass balance modelling

[3] % source type end member samples classified correctly by composite signature

Table A7.67: The final composite signatures selected using KW-H and PCA for the River Burn (Tavy) study catchment.

KW-H			PCA		
Property	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**
Gd	67	1.00	Ce	53	1.20
Ge	69	1.03	Cu	44	1.00
La	67	1.01	Fe	57	1.30
Nd	66	1.00	K	45	1.02
Pr	69	1.04	La	67	1.53
Sm	70	1.05	Y	68	1.54
Total[3]	90		Total[3]	85	

[1] % source type end member samples classified correctly by individual properties

[2] tracer discriminatory weighting used in the mass balance modelling

[3] % source type end member samples classified correctly by composite signature

Table A7.68: The final composite signatures selected using KW-H and PCA for the River East Allen study catchment.

KW-H			PCA		
Property	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**
Bi	39	1.06	Cs	72	2.07
Cs	72	1.96	Cu	39	1.12
Cu	39	1.06	Mg	62	1.79
Dy	37	1.00	Na	57	1.64
Gd	47	1.27	Ni	36	1.04
Mg	62	1.69	Sr	37	1.06
Na	57	1.55	Ti	60	1.71
Sr	37	1.01	V	64	1.84
Ti	60	1.62	Y	40	1.15
V	64	1.75	Zr	35	1.00
Total[3]	92		Total[3]	86	

[1] % source type end member samples classified correctly by individual properties

[2] tracer discriminatory weighting used in the mass balance modelling

[3] % source type end member samples classified correctly by composite signature

Table A7.69: The final composite signatures selected using KW-H and PCA for the River Greta study catchment.

KW-H			PCA		
Property	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**
As	66	1.08	As	66	1.28
Ce	66	1.08	Ba	51	1.00
Fe	74	1.22	Fe	74	1.45
Ga	76	1.26	Mg	66	1.29
Ti	61	1.00	Pb	58	1.13
V	72	1.18	Ti	61	1.18
Total[3]	89		Total[3]	79	

[1] % source type end member samples classified correctly by individual properties

[2] tracer discriminatory weighting used in the mass balance modelling

[3] % source type end member samples classified correctly by composite signature

Table A7.70: The final composite signatures selected using KW-H and PCA for the River South Tyne study catchment.

KW-H			PCA		
Property	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**
As	49	1.24	As	49	1.24
Cd	43	1.10	K	52	1.32
Co	48	1.21	Mg	49	1.25
Cs	52	1.32	Mn	54	1.36
K	52	1.32	Na	40	1.00
Ni	53	1.35	Pb	42	1.07
Rb	55	1.41	Sr	43	1.10
Ti	57	1.45	Ti	57	1.45
Zn	39	1.00	Zn	39	1.00
Total[3]	82		Total[3]	76	

[1] % source type end member samples classified correctly by individual properties

[2] tracer discriminatory weighting used in the mass balance modelling

[3] % source type end member samples classified correctly by composite signature

Table A7.71: The final composite signatures selected using KW-H and PCA for the St Lawrence Stream study catchment.

KW-H			PCA		
Property	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**
Fe	41	1.11	As	41	1.11
Ge	58	1.57	Cu	48	1.28
Mn	37	1.00	Fe	41	1.11
Sb	62	1.67	K	46	1.26
Sr	61	1.64	Li	51	1.37
Tl	44	1.19	Mg	48	1.30
V	50	1.36	Mn	37	1.00
Total[3]	96		Total[3]	80	

[1] % source type end member samples classified correctly by individual properties

[2] tracer discriminatory weighting used in the mass balance modelling

[3] % source type end member samples classified correctly by composite signature

Table A7.72: The final composite signatures selected using KW-H and PCA for the Wye study catchment.

KW-H			PCA		
Property	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**
Al	70	1.05	Al	70	1.12
Lu	67	1.00	K	63	1.00
Rb	69	1.03	Mn	70	1.12
Yb	70	1.05	Zn	67	1.07
Total[3]	86		Total[3]	93	

[1] % source type end member samples classified correctly by individual properties

[2] tracer discriminatory weighting used in the mass balance modelling

[3] % source type end member samples classified correctly by composite signature

Table A7.73: The results of GA-DFA for the Afon Ystwyth study catchment.

GA-DFA 1			GA-DFA 2			GA-DFA 3		
Property	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**
Cs	65	1.26	As	78	1.44	As	78	1.51
Sb	63	1.22	Cs	65	1.20	Cs	65	1.26
Sc	51	1.00	Sb	63	1.16	Hf	60	1.17
Sn	54	1.06	U	54	1.00	Sc	51	1.00
Tl	65	1.28	V	56	1.05	Sn	54	1.06
V	56	1.10	Total[3]	93		V	56	1.10
Total[3]	93					Total3	90	

[1] % source type end member samples classified correctly by individual properties

[2] tracer discriminatory weighting used in the mass balance modelling

[3] % source type end member samples classified correctly by composite signature

Table A7.74: The results of GA-DFA for the Arkle Beck study catchment.

GA-DFA 1			GA-DFA 2			GA-DFA 3		
Property	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**
Cs	60	1.00	Bi	78	1.19	Mo	55	1.08
Er	66	1.10	Er	66	1.00	U	67	1.31
Ho	71	1.19	Ho	71	1.08	V	80	1.56
Yb	71	1.19	Yb	71	1.08	Zn	51	1.00
Total[3]	100		Total[3]	100		Total[3]	100	

[1] % source type end member samples classified correctly by individual properties

[2] tracer discriminatory weighting used in the mass balance modelling

[3] % source type end member samples classified correctly by composite signature

Table A7.75: The results of GA-DFA for the Bedburn Beck study catchment.

GA-DFA 1			GA-DFA 2			GA-DFA 3		
Property	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**
Al	56	1.00	Al	56	1.00	As	67	1.45
Cs	71	1.26	Cs	71	1.26	Cs	71	1.52
Dy	58	1.03	Ga	72	1.28	Gd	53	1.15
Ga	72	1.28	Rb	66	1.17	Na	46	1.00
Rb	66	1.17	Sb	68	1.21	Rb	66	1.41
Sb	68	1.21	Total[3]	97		Total[3]	97	
Total[3]	97							

[1] % source type end member samples classified correctly by individual properties

[2] tracer discriminatory weighting used in the mass balance modelling

[3] % source type end member samples classified correctly by composite signature

Table A7.76: The results of GA-DFA for the Bolingey Stream study catchment.

GA-DFA 1			GA-DFA 2			GA-DFA 3		
Property	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**
Al	47	1.00	Al	47	1.00	La	56	1.31
Gd	61	1.30	Ho	69	1.49	Li	43	1.01
La	56	1.19	La	56	1.19	Nd	54	1.27
Nd	54	1.16	Nd	54	1.16	Sc	43	1.00
V	56	1.20	V	56	1.20	Ti	56	1.31
Total[3]	100		Total[3]	100		Total[3]	100	

[1] % source type end member samples classified correctly by individual properties

[2] tracer discriminatory weighting used in the mass balance modelling

[3] % source type end member samples classified correctly by composite signature

Table A7.77: The results of GA-DFA for the Egglestone Beck study catchment.

GA-DFA 1			GA-DFA 2			GA-DFA 3		
Property	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**
Al	49	1.01	Al	49	1.01	Cs	54	1.39
As	69	1.43	K	49	1.00	Gd	75	1.93
Cs	54	1.11	Sb	56	1.16	Rb	39	1.00
K	49	1.00	Sc	58	1.20	Sb	56	1.45
Sb	56	1.16	Sr	58	1.20	Sr	58	1.50
Sr	58	1.20	Y	60	1.24	V	39	1.00
Total[3]	100		Zr	63	1.29	Y	60	1.55
			Total[3]	100		Total[3]	100	

[1] % source type end member samples classified correctly by individual properties

[2] tracer discriminatory weighting used in the mass balance modelling

[3] % source type end member samples classified correctly by composite signature

Table A7.78: The results of GA-DFA for the Hayle study catchment.

GA-DFA 1			GA-DFA 2			GA-DFA 3		
Property	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**
Ba	40	1.13	Er	37	1.04	Dy	36	1.01
Dy	36	1.01	Li	46	1.31	Er	37	1.04
Er	37	1.04	Lu	39	1.10	Li	46	1.31
Li	46	1.31	Na	70	1.98	Lu	39	1.10
Na	70	1.98	Sb	35	1.00	Na	70	1.98
Sb	35	1.00	Tb	48	1.36	Sb	35	1.00
Tm	41	1.16	Tm	41	1.16	Tm	41	1.16
Yb	36	1.03	Yb	36	1.03	Yb	36	1.03
Total[3]	100		Total[3]	100		Total[3]	100	

[1] % source type end member samples classified correctly by individual properties

[2] tracer discriminatory weighting used in the mass balance modelling

[3] % source type end member samples classified correctly by composite signature

Table A7.79: The results of GA-DFA for the Hudeshope Beck study catchment.

GA-DFA 1			GA-DFA 2			GA-DFA 3		
Property	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**
Al	48	1.24	Cu	45	1.17	Cs	63	1.47
Ga	60	1.55	Ga	60	1.55	Cu	45	1.05
Ni	39	1.00	Ni	39	1.00	Ga	60	1.39
Rb	60	1.56	Rb	60	1.56	Rb	60	1.40
Tm	43	1.12	Tm	43	1.12	Tm	43	1.00
V	59	1.52	V	59	1.52	V	59	1.36
Total[3]	100		Total[3]	100		Total[3]	100	

[1] % source type end member samples classified correctly by individual properties

[2] tracer discriminatory weighting used in the mass balance modelling

[3] % source type end member samples classified correctly by composite signature

Table A7.80: The results of GA-DFA for the Mardle study catchment.

GA-DFA 1			GA-DFA 2			GA-DFA 3		
Property	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**
Al	68	1.67	Ba	61	1.43	Al	68	1.36
Ba	61	1.49	Bi	49	1.15	As	51	1.02
Er	68	1.66	Cd	52	1.22	Ba	61	1.21
Lu	68	1.65	Er	68	1.60	Co	57	1.13
Mg	65	1.59	Eu	55	1.28	Er	68	1.35
Na	41	1.00	Lu	68	1.59	Ga	71	1.40
Rb	68	1.66	Mg	65	1.53	K	50	1.00
Ti	43	1.04	Rb	68	1.60	Mg	65	1.29
Yb	68	1.66	Sr	60	1.40	Rb	68	1.35
Total[3]	100		Ti	43	1.00	Sn	59	1.17
			Tl	69	1.62	Sr	60	1.18
			Yb	68	1.60	Yb	68	1.35
			Total[3]	100		Total[3]	100	

[1] % source type end member samples classified correctly by individual properties

[2] tracer discriminatory weighting used in the mass balance modelling

[3] % source type end member samples classified correctly by composite signature

Table A7.81: The results of GA-DFA for the Nant Magwr study catchment.

GA-DFA 1			GA-DFA 2			GA-DFA 3		
Property	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**
Gd	50	1.24	Eu	44	1.02	Ga	39	1.00
La	41	1.00	Gd	50	1.17	Gd	50	1.28
Mo	67	1.65	Sm	54	1.26	Sm	54	1.38
Sm	54	1.34	Sn	43	1.00	Sn	43	1.09
Tb	56	1.39	Sr	48	1.12	Tb	56	1.43
Y	43	1.06	Y	43	1.00	Tm	45	1.14
Total[3]	94		Total[3]	94		Y	43	1.09
						Total[3]	96	

[1] % source type end member samples classified correctly by individual properties

[2] tracer discriminatory weighting used in the mass balance modelling

[3] % source type end member samples classified correctly by composite signature

Table A7.82: The results of GA-DFA for the Porthleven Stream study catchment.

GA-DFA 1			GA-DFA 2			GA-DFA 3		
Property	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**
Ce	52	1.41	Ce	52	1.43	Cr	37	1.14
Cr	37	1.00	Cr	37	1.02	Er	40	1.23
Eu	39	1.05	Eu	39	1.07	Eu	39	1.19
Gd	47	1.27	Ni	39	1.08	Ho	44	1.35
V	66	1.79	Pr	43	1.17	Nd	36	1.12
Zr	49	1.32	Sm	36	1.00	Tm	33	1.00
Total[3]	100		V	66	1.82	V	66	2.03
			Zr	49	1.34	Total[3]	100	
			Total[3]	100				

[1] % source type end member samples classified correctly by individual properties

[2] tracer discriminatory weighting used in the mass balance modelling

[3] % source type end member samples classified correctly by composite signature

Table A7.83: The results of GA-DFA for the Rea Brook study catchment.

GA-DFA 1			GA-DFA 2			GA-DFA 3		
Property	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**
Al	44	1.00	Al	44	1.13	Al	44	1.00
Ga	54	1.23	Ce	58	1.47	Ce	58	1.30
Ho	54	1.22	Cr	43	1.09	Ga	54	1.23
Lu	62	1.41	Ga	54	1.39	Gd	63	1.43
Nd	64	1.45	Ge	70	1.80	Ge	70	1.59
Sr	62	1.40	Pb	50	1.28	Ho	54	1.22
Ti	53	1.19	Ti	53	1.35	Nd	64	1.45
V	60	1.36	Tm	39	1.00	Sr	62	1.40
Total[3]	98		V	60	1.54	V	60	1.36
			Yb	58	1.48	Yb	58	1.31
			Total[3]	98		Total[3]	98	

[1] % source type end member samples classified correctly by individual properties

[2] tracer discriminatory weighting used in the mass balance modelling

[3] % source type end member samples classified correctly by composite signature

Table A7.84: The results of GA-DFA for the Red Tarn Beck study catchment.

GA-DFA 1			GA-DFA 2			GA-DFA 3		
Property	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**
As	65	1.28	Bi	69	1.47	Bi	69	1.47
Gd	51	1.00	Mo	85	1.80	Mo	85	1.80
Mo	85	1.66	Nd	56	1.18	Nd	56	1.18
Sm	59	1.16	Tb	47	1.00	Tb	47	1.00
Tm	70	1.38	Tm	70	1.49	Tm	70	1.49
Total[3]	100		Zr	72	1.53	Zr	72	1.53
	100		Total[3]	98		Total[3]	98	

[1] % source type end member samples classified correctly by individual properties

[2] tracer discriminatory weighting used in the mass balance modelling

[3] % source type end member samples classified correctly by composite signature

Table A7.85: The results of GA-DFA for the River Burn (Tavy) study catchment.

GA-DFA 1			GA-DFA 2			GA-DFA 3		
Property	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**
Ge	69	1.15	Gd	67	1.47	Gd	67	1.48
Mo	61	1.02	Ge	69	1.51	Ge	69	1.52
Nd	66	1.11	Nd	66	1.46	K	45	1.00
Pr	69	1.16	Pr	69	1.52	Mo	61	1.36
Tl	60	1.00	Sb	45	1.00	Nd	66	1.48
Y	68	1.13	Tl	60	1.32	Pr	69	1.53
Total[3]	100		Total[3]	100		Tl	60	1.33
						Total[3]	100	

[1] % source type end member samples classified correctly by individual properties

[2] tracer discriminatory weighting used in the mass balance modelling

[3] % source type end member samples classified correctly by composite signature

Table A7.86: The results of GA-DFA for the River East Allen study catchment.

GA-DFA 1			GA-DFA 2			GA-DFA 3		
Property	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**
Bi	39	1.29	Bi	39	1.37	As	37	1.13
Cs	72	2.39	Cs	72	2.55	Bi	39	1.18
Dy	37	1.22	Cu	39	1.38	Cs	72	2.19
Er	39	1.29	Dy	37	1.30	Dy	37	1.12
Ho	45	1.49	Ho	45	1.58	Gd	47	1.42
Na	57	1.90	Na	57	2.02	Hf	33	1.00
Sr	37	1.23	Sr	37	1.31	Ho	45	1.36
Ti	60	1.98	Ti	60	2.10	Tb	37	1.12
Y	40	1.33	Tm	28	1.00	Y	40	1.22
Yb	30	1.00	Y	40	1.41	Zr	35	1.06
Total[3]	96		Total[3]	98		Total[3]	96	

[1] % source type end member samples classified correctly by individual properties

[2] tracer discriminatory weighting used in the mass balance modelling

[3] % source type end member samples classified correctly by composite signature

Table A7.87: The results of GA-DFA for the River Greta study catchment.

GA-DFA 1			GA-DFA 2			GA-DFA 3		
Property	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**
Cs	66	1.47	As	66	1.10	Cs	66	1.47
Eu	49	1.11	Bi	60	1.00	Eu	49	1.11
Ge	66	1.47	Ge	66	1.10	Ge	66	1.47
Li	45	1.00	Nd	66	1.10	Li	45	1.00
Mg	66	1.48	V	72	1.20	Mg	66	1.48
Sb	67	1.50	Total[3]	97		Sb	67	1.50
Total[3]	97					Total[3]	97	

[1] % source type end member samples classified correctly by individual properties

[2] tracer discriminatory weighting used in the mass balance modelling

[3] % source type end member samples classified correctly by composite signature

Table A7.88: The results of GA-DFA for the River South Tyne study catchment.

GA-DFA 1			GA-DFA 2			GA-DFA 3		
Property	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**
Cd	43	1.34	As	49	1.51	As	49	1.51
Cs	52	1.60	Cs	52	1.60	Cd	43	1.34
Ga	45	1.39	Ga	45	1.39	Co	48	1.47
Mg	49	1.52	Na	40	1.22	Cs	52	1.60
Mn	54	1.66	Ni	53	1.64	Ga	45	1.39
Na	40	1.22	Zn	39	1.22	Mg	49	1.52
Sc	46	1.42	Zr	32	1.00	Pb	42	1.30
U	53	1.64	Total[3]	90		Sr	43	1.34
Zr	32	1.00				U	53	1.64
Total[3]	92					Zr	32	1.00
						Total[3]	90	

[1] % source type end member samples classified correctly by individual properties

[2] tracer discriminatory weighting used in the mass balance modelling

[3] % source type end member samples classified correctly by composite signature

Table A7.89: The results of GA-DFA for the St Lawrence Stream study catchment.

GA-DFA 1			GA-DFA 2			GA-DFA 3		
Property	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**
Dy	62	1.50	As	41	1.00	As	41	1.00
Fe	41	1.00	Er	51	1.23	Dy	62	1.50
Ho	51	1.24	Gd	57	1.40	Fe	41	1.00
Mg	48	1.17	Ge	58	1.41	Ho	51	1.24
Nd	57	1.39	Nd	57	1.39	Lu	52	1.28
Sb	62	1.51	Sb	62	1.51	Pr	58	1.40
Sr	61	1.48	Tb	50	1.21	Sb	62	1.51
V	50	1.23	Total[3]	100		Total[3]	100	
Total[3]	100							

[1] % source type end member samples classified correctly by individual properties

[2] tracer discriminatory weighting used in the mass balance modelling

[3] % source type end member samples classified correctly by composite signature

Table A7.90: The results of GA-DFA for the Wye study catchment.

GA-DFA 1			GA-DFA 2			GA-DFA 3		
Property	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**	**Property**	**%[1]**	**TDW[2]**
Al	70	1.12	Al	70	1.12	Al	70	1.12
Er	70	1.12	K	63	1.00	Er	70	1.12
K	63	1.00	Lu	67	1.07	K	63	1.00
Rb	69	1.09	Mn	70	1.12	Rb	69	1.09
Total[3]	93		Total[3]	93		Total[3]	93	

[1] % source type end member samples classified correctly by individual properties

[2] tracer discriminatory weighting used in the mass balance modelling

[3] % source type end member samples classified correctly by composite signature